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REMARKS/ARGUMENTS

With this amendment, claims 1, 4-6, and 15-25 are pending. Claims 7-13 are withdrawn. Claims 2, 3, and 14 are cancelled without prejudice to subsequent revival. New claims 18-25 are added. For convenience, the Examiner's rejections are addressed in the order presented in a February 18, 2004 Office Action.

I. Status of the claims

Claims 2, 3, and 14 are cancelled without prejudice to subsequent revival.

Claim 1 is amended to recite a method of production of a recombinant expression product using semi-continuous culture of transgenic plant cells that comprise an expression cassette that includes promoter induced by sugar depletion to regulate expression of the recombinant product. Support for this amendment is found throughout the specification, for example, at page 7, lines 32-34; at page 12, lines 15-26; and at page 13, lines 1-14. The method includes the step of exchanging an induction medium with a growth medium after the induction medium pH increases. Support for this amendment is found throughout the specification, for example, at page 4, lines 32-33 and at page 26, lines 22-26. These amendments add no new matter.

Claim 16 is amended to recite that the expression product is a human α_1-antitrypsin protein. Support for this amendment is found throughout the specification, for example, at page 4, lines 24-26. This amendment adds no new matter.

New claims 18 and 19 depend from claim 1 and recite exchanging the induction medium with the growth medium when the pH of the induction medium is above pH 5.5 or 6.0 respectively. Support for these amendments is found throughout the specification, for example, at page 5, lines 2-6. These amendments add no new matter. Applicants also point out that withdrawn claims 6 and 7 recite medium exchange at pH values above 6.5 and 7.0, respectively.

New claim 20 depends from claim 1 and recites the additional step of isolating the recombinant expression product from the induction medium. Support for this amendment is

found throughout the specification, for example, at page 16, lines 9-10. This amendment adds no new matter.

New claim 21 depends from claim 1 and recites that the promoter is a cereal amylase promoter. Support for this amendment is found throughout the specification, for example, at page 12, line 17. New claim 22 depends from claim 1 and recites that the promoter can be selected from any of the following promoters: RAmy1A promoter, a RAmy1B promoter, a RAmy2A promoter, a RAmy3A promoter, a RAmy3B promoter, a RAmy3C promoter, a RAmy3D promoter, a RAmy3E promoter, an αAmy8 promoter, a pM/C promoter, a gKAmy141 promoter, a gKAmy155 promoter, an Amy32b promoter, and a barley HV18 α-amylase promoter. Support for this amendment is found throughout the specification, for example, at page 12, lines 20-25 and at page 13, line 3. New claim 23 depends from claim 1 and recites that the promoter is a rice α amylase promoter. Support for this amendment is found throughout the specification, for example, at page 13, line 2. New claim 24 depends from claim 1 and recites that the promoter is a RAmy3D promoter. Support for this amendment is found throughout the specification, for example, at page 12, line 21 and at page 13, line 3. New claim 25 depends from claim 1 and recites that the promoter is an αAmy8 promoter. Support for this amendment is found throughout the specification, for example, at page 13, line 3. These amendments add no new matter.

II. Rejections under 35 U.S.C. §112, first paragraph, enablement

Claims 1-6 and 14-17 are rejected under 35 U.S.C. §112, first paragraph because, allegedly, the specification does not enable the full scope of the claims. The Office Action does allow that the specification is enabling for a method of semi-continuous growth of rice suspension cells transformed with the *RAmy3D* promoter operably linked to a human α_1-antitrypsin encoding nucleic acid. To the extent the rejection applies to the amended claims, Applicants respectfully traverse the rejection.

The Examiner appears to have focused improperly on inoperative embodiments, leading to the conclusion that undue experimentation would be required to identify expression products, inducible promoters, and plant cells for use in the claimed invention. However, the

proper test of enablement is "whether one skilled in the art could make or use the claimed invention from the disclosure in the patent coupled with information known in the art without undue experimentation" (*see, e.g.*, MPEP §2164.01). In the present application, one of skill would know how to avoid inoperative embodiments and how to produce recombinant protein in plant cells under the control of a promoter induced by sugar depletion by exchanging induction medium after a pH increase, without undue experimentation (*see, In re Cook and Merigold*, 169 USPQ 299, 301 (C.C.P.A. 1971)). Moreover, the present application provides guidance in the form of assays and working examples for determining pH values and expression product levels in induction medium.

Claims reading on inoperative embodiments are enabled if the skilled artisan understands how to avoid inoperative embodiments. As described by the court in *In re Cook and Merigold*, 169 USPQ 302:

> Many patented claims read on vast numbers of inoperative embodiments in the trivial sense that they can and do omit 'factors which must be presumed to be within the level of ordinary skill in the art'....There is nothing wrong with this so long as it would be obvious to one of ordinary skill in the relevant art how to include those factors in such a manner as to make the embodiment operative rather than inoperative.

See, In re Cook and Merigold, 169 USPQ at 302 (quoting in part *In re Skrivan*, 166 USPQ 85, 88 (C.C.P.A. 1970)).

The amended claims are directed to a method of production of a recombinant protein using semi-continuous culture of transgenic plant cells that comprise an expression cassette that includes a promoter induced by sugar depletion that regulates expression of the recombinant protein. The method includes the step of exchanging an induction medium with a growth medium after the pH of the induction medium increases.

Factors such as the amount of guidance presented in the specification and the presence of working examples must be considered to determine whether undue experimentation is required to practice the claimed invention (*see, Ex Parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int. 1985) and *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988)). As described in *Wands*,

"a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed" (*see*, *Wands*, USPQ2d at 1404, quoting *In re Jackson*, 217 USPQ 804 (Bd. Pat. App. & Int. 1982)).

The specification also provides standard assays and working examples for determining expression of proteins and for measuring pH of an induction medium. (See, *e.g.*, specification at page 15, lines 1-32 and page 8, lines 21-25 and lines 30-33.) Those of skill in the art would clearly be able to use the specification to identify appropriate conditions and practice the methods of the invention for expression of recombinant proteins in plant cells. Identification of operable embodiments, therefore, is well within the means of one of skill of the art, without undue experimentation.

The Office Action also alleges that the claimed methods are not enabled for plant cells other than rice, for products other than recombinant human α_1-antitrypsin protein, or for promoters other than *RAmy3D*. According to the Office Action, those of skill could not predict which culturing conditions apply to plant cells of any particular species and that undue experimentation would be required to identify an operable promoter-plant cell combination. In addition, the Office Action asserts that the methods are unpredictable because the ionic nature of expression products vary and thus, would allegedly influence the pH of the culture.

The Office Action is incorrect. Applicants submit as Exhibit A, a declaration from inventor Dr. Karen McDonald providing evidence that the specification teaches methods of recombinant protein production from plant cells under control of a promoter induced by sugar depletion, using a pH increase to monitor recombinant protein production, and thus, enables the claimed methods.

First, Dr. McDonald asserts that the pH increase in the induction medium that coincides with the increased production levels of human α_1-antitrypsin did not result from the increased expression protein levels in the media. Dr. McDonald points out that the pH increases ranged from lower values of 4.4-5.1 to higher values of 7.0-7.4. Dr. McDonald also states that human α_1-antitrypsin is an acidic protein with a pI of 5.4. In her opinion, it is unlikely that addition of a protein with a pI of 5.4 would raise the pH of the media from *e.g.*, 5.1 to 7.0-7.4.

Thus, the pH increase was not the result of over production of human α_1-antitrypsin. In addition, the declaration provides evidence that a pH increase occurs in the induction medium even when rice cells that do not produce a recombinant protein are grown under the semi-continuous culture conditions described in the specification. In Dr. McDonald's opinion, overexpression of a majority of proteins will not result in medium pH changes, but will coincide with an increase in induction medium pH, as is seen for overexpression of human α_1-antitrypsin.

Dr. McDonald also states that promoters induced by sugar depletion can be used for high level production of many different proteins. For example, the *RAmy3D* promoter has been used to express GUS protein and human GMCSF, as well as the exemplified human α_1-antitrypsin, in rice cells. (See, *e.g.*, Chan *et al.*, *J. Biol. Chem.* 269:17635 (1994), submitted as Exhibit B, and Shin *et al.*, *Biotech. & Bioeng.* 82:778-783 (2003), submitted as Exhibit C.) In addition, the *RAmy8* promoter has been used to express high levels of protein in tobacco cell, rice cells, and potato cells, indicating that sugar regulated promoters can be used in a variety of plant tissue culture cells. (See, *e.g.*, Chan *et al.*, Figure 4.) In Dr. McDonald's opinion, expression of recombinant proteins in plant cells under control of a promoter induced by sugar depletion, can be monitored by following pH levels of the induction medium. Dr. McDonald also believes that the teachings of the specification can be used by those of skill to determine pH values that coincide with recombinant protein expression under control of a promoter induced by sugar depletion in a plant cell.

In view of the above amendments and remarks, Applicants respectfully request withdrawal of the rejection.

III. Rejections under 35 U.S.C. §103(a)

Claims 1-6 and 14-17 are rejected under 35 U.S.C. §103(a) as allegedly obvious in view of Terashima *et al.* (*Appl. Microbiol. Biotechnol.* 52:516-523 (1999)) in view of Fischer *et al.* (*Plant Cell, Tissue and Organ Culture* 38:123-134 (1994)).

To establish a *prima facie* case of obviousness, three basic criteria must be met: (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to

combine reference teachings; (2) there must be a reasonable expectation of success; and (3) the prior art reference must teach or suggest all the claims limitations. MPEP§2143. See also *In re Rouffet,* 47 USPQ2d 1453. The court in *Rouffet* stated that "even when the level of skill in the art is high, the Board must identify specifically the principle, known to one of ordinary skill, that suggests the claimed combination." *Rouffet* at 1459. The court has also stated that actual evidence of a suggestion, or teaching, or motivation to combine is required and the showing of a suggestion, or teaching, or motivation to combine must be "clear and particular." *In re Dembiczak*, 50 USPQ2d 1614, 1617 (1999).

The references cited by the Examiner fail to provide a reasonable expectation of success in practicing the invention and fail to provide a motivation for the combination of the references. In addition, the references cited by the Examiner fail to provide all the elements of the rejected claims.

The pending claims are directed to methods of using plant cells to produce recombinant proteins encoded by a nucleic acid under control of a promoter induced by sugar depletion, using induction medium pH values (*e.g.*, an increase in pH) to determine when to exchange the induction medium for growth medium. Neither of the cited references teaches or suggests exchange of induction medium after the pH of the induction medium increases.

According to the Office Action, Terashima *et al.* teaches continuous culture of rice cells expressing recombinant human α_1-antitrypsin. The Office Action admits that Terashima *et al.* does not teach semi-continuous culture conditions, monitoring the pH of the medium, or monitoring the oxygen uptake of the medium. Applicants respectfully point out that the Office Action incorrectly asserts that Terashima *et al.* teach continuous culture of rice cells, when, in fact, the reference teaches batch culture of rice cells with a single medium exchange; cells were not reused for subsequent production of the recombinant protein.

According to the Office Action, Fischer *et al* teach semi-continuous production of photoautotrophic plant cells. However, Fischer *et al.* does not disclose or suggest medium exchanges based on pH or protein expression. In fact, Fischer *et al.* show only that a number of variables, such as pH, dissolved O_2 and dissolved CO_2, did not vary with cellular protein content

under culture conditions optimized for photoautotrophic cell suspension cultures. *See, e.g.*, Figures 3 and 4.

The Office Action also alleges that statements in the application constitute an admission of knowledge in the art of the claimed methods or of a motivation in the art to combine the teaching of Terashima *et al* with Fischer *et al*. This allegation is erroneous. The paragraph referred to by the Office Action was taken out of context. The inventors were the first to demonstrate that an increase in pH levels after a switch to induction medium coincides with the expression of recombinant protein in the induction medium. The cited statement refers back to an earlier paragraph and states that, after the correlation between an increase in induction medium pH and an increase in level of recombinant protein in the medium was established by the inventors, those of skill would be able to use that correlation under a variety of conditions, *e.g.*, by monitoring pH and protein expression to determine the appropriate pH value for medium exchange. However, the correlation between a pH increase in induction medium and recombinant protein levels was discovered by the inventors and was not known to those of skill in the art prior to the filing of this application.

Neither reference provides motivation to assay pH for correlation with recombinant protein production in semi-continuous culture of transgenic plant cells. Terashima *et al*. emphasize the correct post-translational processing of the recombinant protein, the speed of protein production compared to other plant systems, and the advantages of the rice cell tissue culture over mammalian or microbial expression systems. (Terashima *et al*., pages 521-522.) Terashima *et al*. does not provide motivation for measurement of pH levels to determine protein expression levels or to improve their production methods.

Fischer *et al* does not teach recombinant protein production, and in fact demonstrates that cellular protein production and some medium values, *e.g.*, dissolved O_2, dissolved CO_2, and pH values, do not vary during semi-continuous culture of photoautotrophic plant cells. Fischer does not provide any motivation for selecting any medium values, including pH, for correlation of recombinant protein expression levels in induction medium.

Finally, any 'admission' perceived by the Examiner in the application is in error, as discussed above, and cannot be used to find motivation for measurement of pH levels to

determine protein expression levels in the knowledge of those of skill in the art or, in fact, outside the teaching of the specification.

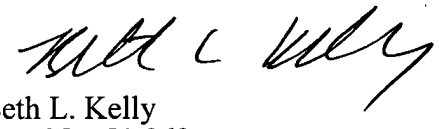
In view of these arguments, Applicants respectfully request withdrawal of the rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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Attachments
BLK:
60151050 v1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

McDonald, Karen A., *et al.*

Application No.: 09/992,845

Filed: November 14, 2001

For: PROCESS FOR SCALED-UP PRODUCTION OF RECOMBINANT PROTEINS USING TRANSGENIC PLANT SUSPENSION CULTURES

Customer No.: 20350

Confirmation No. 7257

Examiner: Russell Kallis

Technology Center/Art Unit: 1638

DECLARATION UNDER 37 C.F.R § 1.132 OF DR. KAREN A. MCDONALD

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Karen A. McDonald, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I received a Masters Degree in chemical engineering from the University of California, Berkeley in 1980. I was on the technical staff at Sandia National Laboratories from 1979 to 1982. I received a Ph.D. in chemical engineering from the University of Maryland in 1985. I joined the faculty of the Department of Chemical Engineering and Materials Science at the University of California, Davis as an assistant professor in 1985. I was promoted to associate professor in 1993. In 1998, I became a full professor in the Department of Chemical Engineering and Materials Science and continue to hold that position. In 2000, I was appointed Acting Associate Dean of Research and Graduate Studies of the College of Engineering at the University of California, Davis. Since 2001, I have held the position of Associate Dean of

Exhibit A

Research and Graduate Studies of the College of Engineering at the University of California, Davis. A copy of my curriculum vitae is attached hereto as Exhibit D.

3. The present application provides methods of using plant cells to produce recombinant proteins encoded by a nucleic acid under control of a promoter induced by sugar depletion, using induction medium pH values (*e.g.*, an increase in medium pH) to determine the time of medium exchange.

4. I am a named inventor on the above-referenced patent application. I have read and am familiar with the contents of the patent application. In addition, I have read the Office Action, mailed February 18, 2004, received in the present case. It is my understanding that the Examiner is concerned that the claimed methods are not enabled by the specification. Specifically, the Examiner states that the specification is enabling for a method of semi-continuous growth of rice suspension cells transformed with the *RAmy3D* promoter operably linked to a human α_1-antitrypsin encoding nucleic acid. (Office action at page 2.) However, the Examiner states that the specification does not reasonably provide enablement for a method of semi-continuous growth of any plant cell suspension culture expressing any protein under the control of any inducible promoter.

5. This declaration is provided to demonstrate that practice of the claimed methods is fully enabled by the specification. This declaration provides evidence that pH increases in the induction medium, while coincident with expression of the recombinant protein, are not the direct result of the influence of a particular recombinant protein on the induction medium pH, as asserted by the Office Action. This declaration also provides evidence that the promoters induced by sugar depletion can be used to induce expression of a variety of proteins in a variety of cell types. One of skill in the art can, therefore, practice the claimed methods using information provided in the specification, together with methodology known to one of skill in the art, with at most, only routine experimentation.

6. The specification provides examples of production of the human α_1-antitrypsin protein by rice cells transformed with a nucleic acid encoding the protein under the

control of the *RAmy3D* promoter. After addition of an induction medium that is free of metabolizable carbohydrates to induce production of the human α_1-antitrypsin protein, the pH of the induction medium increased, ranging from low values of 4.4-5.1 to high values of 7.0-7.4. (Specification at page 26, lines 25-26, results of three separate experiments.) Human α_1-antitrypsin is an acidic protein with a pI of 5.4 and a molecular weight of about 52,000 daltons. The three experiments resulted in production of 40, 110, and 80 milligrams of human α_1-antitrypsin protein per liter of induction medium. (Specification at Table 3, page 25.) The molar concentration of human α_1-antitrypsin protein thus ranged from 0.77 μM to 2.11 μM. In my opinion, it is unlikely that addition of a protein with a pI of 5.4 at concentrations from 0.77 μM to 2.11 μM would raise the pH of the media from *e.g.*, 5.1 to 7.0-7.4. Thus, the observed pH increase was not the direct result of the "ionic nature of the expression product" as alleged by the Office Action at page 4. In my opinion, by using a promoter induced by sugar depletion, overexpression of most recombinant proteins in plant cells will coincide with an increase in induction medium pH, as is demonstrated in the specification for overexpression of human α_1-antitrypsin.

7. This declaration provides additional evidence that expression of a particular recombinant protein does not affect the pH of the medium. The attached Figure 1 provides results of an experiment where untransformed rice tissue culture cells were subjected to the growth/induction cycles exemplified in the application. After each switch from growth to induction medium the pH of the induction medium increased. This pH increase occurred in the absence of recombinant protein production. This result provides evidence that expression of a recombinant protein from a plant cell, while correlating with the observed pH increase in induction medium, will not affect or cause the pH increase. Thus, it is my belief that overexpression of a majority recombinant proteins in plant cells under the control of a promoter induced under conditions of sugar depletion will coincide with an increase in induction medium pH, as is demonstrated for overexpression of human α_1-antitrypsin.

8. Promoters that are induced by sugar depletion can be used in the claimed invention. For example, the family of rice α-amylase promoters are induced by sugar starvation and can be used for high level production of many different proteins. The *RAmy8* promoter has

been used to express GUS protein and human α_1-antitrypsin in rice cells, and the *RAmy3D* promoter has been used to express human GMCSF, as well as the exemplified human α_1-antitrypsin, in rice cells. (See, *e.g.*, Chan *et al.*, *J. Biol. Chem.* 269:17635 (1994), submitted as Exhibit B, and Shin *et al.*, *Biotech. & Bioeng.* 82:778-783 (2003), submitted as Exhibit C.) In addition, the RAmy8 promoter has been used to express high levels of protein in tobacco cells, rice cells, and potato cells, indicating that the inducible sugar regulated promoters can be used in a variety of plant tissue culture cells. (See, *e.g.*, Chan *et al.*, Figure 4.) In my opinion, expression of recombinant proteins in plant cells from a nucleic acid using a promoter that is induced by sugar depletion, can be optimized using pH measurement (*e.g.*, a pH increase) in plant cells. In addition, the teachings of the specification can be used by those of skill to determine pH values that coincide with recombinant protein expression under control of a promoter induced by sugar depletion in a plant cell, and thus to practice the claimed methods.

9. In view of the foregoing, it is my scientific opinion that one of skill in the art would be able to practice the claimed invention with, at most, routine experimentation. The specification, therefore, fully enables the methods of the invention.

Date: July 15, 2004 By: Karen A. McDonald

Karen A. McDonald, Ph.D.

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Novel Gene Expression System for Plant Cells Based on Induction of α-Amylase Promoter by Carbohydrate Starvation*

(Received for publication, December 10, 1993, and in revised form, March 25, 1994)

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The 5′ regulatory region and putative signal sequence of a rice α-amylase gene, *αAmy8*, was fused to a bacterial gene encoding β-glucuronidase (GUS) and introduced into rice, tobacco, and potato via *Agrobacterium*-mediated transformation systems. Expression of this chimeric gene in suspension cells of transgenic plants was suppressed by the presence of sucrose in the medium and induced by its absence. Induction or suppression of GUS expression in transgenic rice could be reversed by the deprivation or replenishment, respectively, of sucrose in the medium. The expressed GUS fusion protein was translocated to the endoplasmic reticulum, modified by glycosylation, and secreted into the culture medium of transgenic cells. In the presence of a glycosylation inhibitor, tunicamycin, the enzymatically active form of GUS was assembled in the endoplasmic reticulum. The yield of GUS secreted by transgenic cells was estimated to be as high as 40% of total secreted proteins. The reversible induction of the α-amylase promoter in culture cells by sugar level in the medium provides an excellent inducible expression system for basic research in plant science. Combination of the α-amylase promoter and signal sequence also offers a novel approach for large scale production of low cost, easily purified, secreted recombinant proteins.

Repression by carbon catabolites is a fundamental and ubiquitous regulatory system in both prokaryotic and eukaryotic cells. In bacteria and yeasts, catabolite-regulated gene expression is an essential mechanism for adjusting to changes in nutrient availability (Carlson, 1987; Schuster, 1989; Vyas *et al.*, 1988). In higher plants, carbon metabolite regulation of gene expression plays a fundamentally important role in maintaining an economical balance between supply and demand for biomolecules in various tissues or cells. End products of a metabolic pathway often inhibit the expression of enzymes involved in the pathway. α-Amylases are endoamylolytic enzymes that catalyze the hydrolysis of α-1,4-linked glucose polymers and are important in the degradation of starch in higher plants. Sugars are found to suppress the expression of α-amylase genes in suspension-cultured cells (Yu *et al.*, 1991, 1992) as well as in germinating seeds (Karrer and Rodriguez, 1992) of rice. Our laboratory has been studying the mechanisms of metabolic regulation of α-amylase gene expression in rice. Studies with suspension cell cultures reveal that both the transcription rate and mRNA stability are significantly reduced when cells are grown in medium containing sucrose (Sheu *et al.*, 1994).

Since the repression of α-amylase gene expression in cells grown with sucrose is partly exerted at the transcriptional level, transcriptional activity of the α-amylase promoters should presumably be elevated when cells are grown in medium lacking carbon sources. In this regard the rice α-amylase promoters are similar to the yeast *GAL* promoters in that their transcription is regulated by carbon sources in the medium. The glucose-repressible *GAL* promoters are capable of promoting high-level regulated gene expression when yeast cells are grown in medium containing galactose (Johnston and Davis, 1984) and have been commonly used for inducing gene expression in yeasts. Inducible promoter systems are very useful for controlling the levels of expression of transfected genes for basic studies of the cell's biological effects of a given gene product. The *GAL* and α-amylase promoters may share the common advantage that conditional expression of a gene of interest can be controlled by manipulation of sugar level in the medium, especially when continuous overexpression of the desired product is deleterious or unwanted. One of the goals of the present research was to determine whether the α-amylase promoter is inducible by sugar starvation and is functional in dicotyledonous plant cells as well as in monocotyledonous rice cells. If effective, such a system would be useful for achieving regulated expression of genes introduced into plant cells by gene transfer.

Previously we demonstrated that a 1.2-kb[1] DNA fragment containing the 5′ region of a rice α-amylase gene, *αAmy8*, directed the expression of GUS in all cell types of mature leaves, stems, sheaths, and roots of transgenic rice (Chan *et al.*, 1993). In this report, we show that expression of the *αAmy8* (1.2-kb)/GUS chimeric gene in suspension-cultured transgenic rice cells was suppressed by sugars present in the medium. Expression of the same chimeric gene in transgenic tobacco and potato cells was similarly regulated by sugar. These studies suggest that the carbohydrate starvation-inducible α-amylase promoter is potentially useful for the design of inducible gene expression systems for different plant species. In addition, because of this 1.2-kb DNA fragment containing a predicted N-terminal 25-amino acid signal sequence, GUS was secreted into the culture medium in large quantities from these suspension-cultured transgenic plant cells. The secreted GUS was glycosylated, suggesting that the α-amylase signal sequence has directed the target of GUS into the endoplasmic reticulum (ER). The high inducibility of the α-amylase promoter by carbohydrate starvation in combination with the secretory function of the signal peptide of α-amylase also provides a novel, highly effective expression system for the production of valuable recombinant proteins in plant cell culture.

EXPERIMENTAL PROCEDURES

Plant Materials and Transformation—Rice varieties *Oryza sativa* L. cv. Tainung 62 and *Oryza sativa* L. cv. Tainan 5, potato variety *Solanum*

* This work was supported by Grant NSC82-0418-B001-102-B03 from the National Science Council of the Republic of China. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: kb, kilobase(s); GUS, β-glucuronidase; ER, endoplasmic reticulum; MES, 2-(*N*-morpholino)ethanesulfonic acid; TM, tunicamycin.

Exhibit B

tuberosum L. cv. ADH69, and tobacco variety *Nicotiana tabacum* L. cv. Petit Havana SRI were used in this study. Transgenic rice cell lines were obtained by transformation of immature rice embryos with *Agrobacterium* as previously described (Chan *et al.*, 1993). Transgenic tobacco cell lines were obtained by transformation of leaf discs with *Agrobacterium* according to the method of Horsch *et al.* (1988). Transgenic potato cell lines were obtained by transformation of microtubers with *Agrobacterium*. Microtubers were obtained from *in vitro* cultured potato plants (Wang and Hu, 1982) and sliced into discs of 2-mm thickness. Discs were co-cultured with 25 μl of *Agrobacterium* culture in a Petri dish containing 10 ml of MS liquid medium (Murashige and Skoog, 1962) and then incubated at 26 °C in the dark for 2 days. The infected microtuber discs were blotted dry on sterile filter papers and transferred to solid potato suspension culture medium (each liter contains MS salts, 1 g of casein hydrolysate, 40 mg of adenine sulfate, 0.4 mg of gibberellin A_3, 3 mg of 2,4-dichlorophenoxyacetic acid, 0.3 mg of kinetin, 100 mg of myo-inositol, 0.5 mg of thiamine HCl, 0.5 mg of pyridoxine HCl, 5 mg of nicotinic acid, 2 mg of glycine, 0.05 mg of biotin, 0.05 mg of folic acid, 20 g of sucrose, 1.8 g of glucose, 0.8 g of MES, pH 5.7–5.9, and 8 g of agarose) containing 500 mg/liter cefotaxime and 100 mg/liter kanamycin and incubated under 300 lux illumination for selection of transformants. The tissues were subcultured at weekly intervals. After several weeks, the calli formed from the inoculated microtuber discs were transferred to liquid potato suspension culture medium, shaken on a reciprocal shaker at 120 rpm, and incubated at 25 °C under 300 lux illumination. Suspension cell cultures of the transgenic cell lines were propagated as previously described (Yu *et al.*, 1991).

For induction of GUS production, 1 ml (approximately 0.4 g of cells, fresh weight) of suspension-cultured cells of transgenic cell lines were transferred from sucrose-containing MS medium to 50 ml of sucrose-free MS medium in a 250-ml flask, shaken on a reciprocal shaker at 120 rpm, and incubated at 25 °C in the dark for 48 h. Suspension cells were collected by filtration through a 400-mesh nylon sieve, blot-dried on paper towels, and weighed. Medium was collected by filtration through Whatman No. 1 filter papers. The medium or collected cells were quick-frozen in liquid N_2 and stored at −70 °C until use.

Plasmids and Bacterial Strains—Construction of plasmid pAG8 carrying a chimeric *αAmy8* promoter/GUS (*uidA*) gene has been previously described in detail (Chan *et al.*, 1993). The DNA and amino sequence at the junction of α-amylase and GUS is shown in Fig. 1. Plasmid *αAmy8-C* carries a 1.4-kb α-amylase cDNA insert in pBluescript KS⁺ (Stratagene) (Yu *et al.*, 1992). Generation of the *Agrobacterium tumefaciens* strain A281 (pTiBo542 + pAG8) has been previously described (Chan *et al.*, 1993).

Southern Blot Analysis—Total DNA was isolated from suspension cells of putative transgenic cell lines. DNA blot analysis was performed as described by Maniatis *et al.* (1982). The GUS DNA used as a probe was made from the *Bam*HI-*Sst*I restriction fragment of plasmid pBI221 (Clontech) and labeled with [α-^{32}P]dCTP using the random primer method (Feinberg and Vogelstein, 1983).

Northern Blot Analysis—Total RNA was purified from suspension cells according to the method of Belanger *et al.* (1986). RNA blot analysis was performed as described by Thomas (1983). The 1.4-kb α-amylase cDNA insert used as the probe was excised from plasmid *αAmy8-C* by restriction enzyme *Eco*RI and labeled with [α-^{32}P]dCTP using the random primer method (Feinberg and Vogelstein, 1983).

GUS Activity Assay and Western Blot Analysis—Total proteins were extracted by grinding 0.1–0.2 g of cells in 500 μl of GUS extraction buffer (50 mM sodium phosphate buffer, pH 7.0, 10 mM EDTA, 0.2% Triton X-100, 0.1% Sarkosyl, 10 mM β-mercaptoethanol). The GUS activity assay was done as described by Jefferson (1988). The Western blot analysis was performed as previously described (Yu *et al.*, 1991). The α-amylase polyclonal antibodies against the partially purified rice α-amylases from the culture medium were raised in rabbits. The GUS polyclonal antibodies were purchased from Molecular Probes, Inc.

Protein Dot-blot Analysis—Total proteins extracted by the GUS extraction buffer were spotted onto a nitrocellulose filter using a dot-blot system (Life Technologies, Inc., Hybri-Dot Manifold). GUS was detected by the GUS polyclonal antibodies according to procedures for Western blot analysis.

RESULTS

Transformation of Rice, Tobacco, and Potato with αAmy8/GUS—Plasmid pAG8 was introduced into rice, tobacco, and potato cells via *Agrobacterium*-mediated transformation. Rice cell lines C7 and C11 (both from cv. Tainung 62) and C51 and C52 (both from cv. Tainan 5), tobacco cell lines TO1 and TO2, and potato cell lines P1 and P2 were all randomly selected putatively transgenic cell lines. Integration of the chimeric *αAmy8*/GUS gene into these cell lines was demonstrated by Southern blot analysis of their *Hin*dIII-digested genomic DNA using the GUS DNA from pBI221 as a probe (Fig. 2). DNA fragments containing GUS DNA, which were longer than the 2.3-kb *Hin*dIII-*Bam*HI *αAmy8*/GUS DNA fragment in pAG8, were detected in the genome of rice cell lines C7 and C11 (Fig. 2*A*, *lanes 2* and *3*), C51 and C52 (Fig. 2*B*, *lanes 2* and *3*), tobacco cell lines TO1 and TO2 (Fig. 2*B*, *lanes 5* and *6*), and potato cell lines P1 and P2 (Fig. 2*B*, *lanes 8* and *9*). In addition, the GUS DNA probe did not hybridize to the DNA of the non-transformed cell lines (Fig. 2*A*, *lane 4* and Fig. 2*B*, *lanes 1*, *4*, and *7*). These results suggest that GUS DNA was integrated into the genomes of the transformed cell lines. Tobacco cell line TO2 appeared to have two integration sites for the GUS gene as two hybridization bands were detected (Fig. 2*B*, *lane 6*). The differences in intensity of hybridized bands between individual rice cell lines (C7 and C11, C51 and C52) and between individual potato cell lines (P1 and P2) were probably due to unequal DNA loading. Application of an inverse polymerase chain reaction technique developed for estimating the tDNA copy number in transgenic plants (Does *et al.*, 1991) revealed that except for cell line TO2, all of these transgenic cell lines contained only one integrated tDNA copy (data not shown).

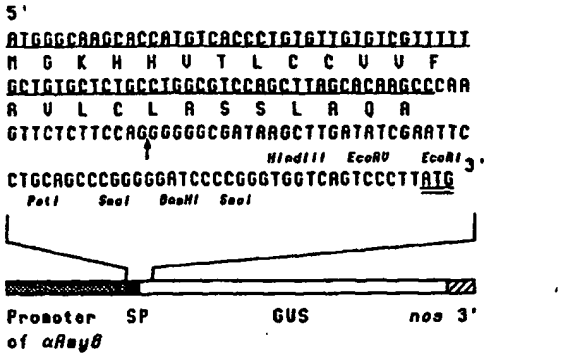

FIG. 1. **DNA and amino acid sequence at the junction of α-amylase and GUS.** Construction of the binary vector pAG8 containing the 1.2-kb 5′ regulatory region of the α-amylase gene *αAmy8*, the coding region of GUS, and the polyadenylation signals of nopaline synthase gene (*nos*) has been previously described (Chan *et al.*, 1993). *Underline* indicates the nucleotide and amino acid sequence of the putative *αAmy8* signal peptide (*SP*) coding region in pAG8. *Arrow* indicates the junction between the α-amylase coding region and the multiple cloning sites and GUS coding region in vector pBSI (a gift from Dr. Ray Wu, Cornell University, Ithaca). *Double underline* indicates the start of the GUS coding region.

Expression of αAmy8/GUS in Transgenic Cell Lines at mRNA Level—To determine whether the expression of *αAmy8*/GUS in transgenic rice, tobacco, and potato cells is regulated by sucrose in the medium, Northern blot analysis was performed with total RNA isolated from cultured suspension cells of transgenic cell lines using the GUS DNA as a probe. The concentration of GUS mRNA was very low in cells grown with sucrose (Fig. 3*A*, *lanes 1* and *3* and Fig. 3*B*, *lanes 3*, *7*, and *11*) but increased significantly after cells were starved of sucrose for 48 h (Fig. 3*A*, *lanes 2* and *4* and Fig. 3*B*, *lanes 4*, *8*, and *12*). No GUS mRNA was detected in the non-transformed control cells regardless of the presence or absence of sucrose in the medium (Fig. 3*A*, *lanes 5* and *6* and Fig. 3*B*, *lanes 1*, *2*, *5*, *6*, *9*, and *10*). Accumulation of the endogenous α-amylase mRNA in C7 and C11 cells was also determined using the α-amylase DNA as a probe. Levels of α-amylase mRNA in C7 and C11 cells and in the non-transformed control cells were low when cells were

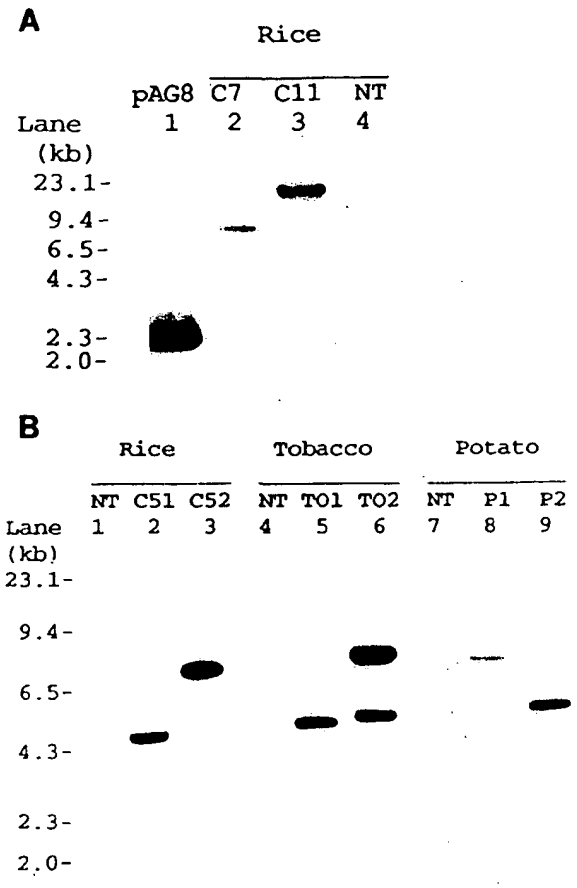

FIG. 2. **Southern blot analysis for detection of GUS DNA in transgenic cell lines.** Genomic DNA was isolated from suspension cells of the putative transgenic plants. Five μg of DNA was digested with *Hin*dIII and hybridized with the ^{32}P-labeled GUS DNA probe. *A*, DNA from two putative transgenic cell lines (C7 and C11) of rice cv. Tainung 62. DNA of plasmid pAG8 was digested with *Hin*dIII and *Bam*HI. *B*, DNA from putative transgenic cell lines of rice cv. Tainan 5 (C51 and C52), tobacco (TO1 and TO2), and potato (P1 and P2). *NT* indicates DNA of the non-transformed control cell lines.

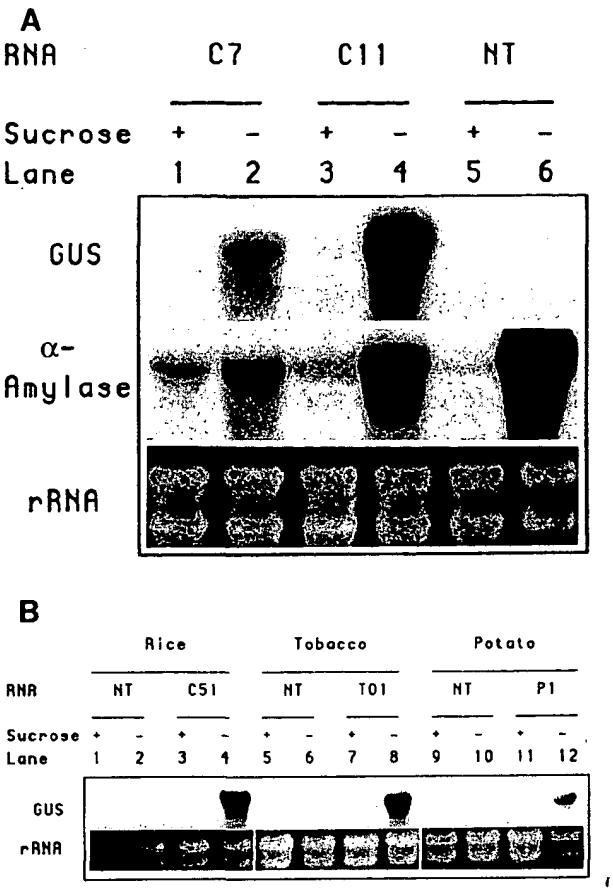

FIG. 3. **Sucrose suppression of GUS gene expression in suspension cells of transgenic plants.** Suspension cells were grown in sucrose-containing medium for 5 days and transferred to sucrose-containing or sucrose-free medium for 2 days. Total RNA was isolated from the suspension cells, subjected to Northern blot analysis, and hybridized with the ^{32}P-labeled GUS or α-amylase DNA probes. Five μg of total RNA was loaded in each *lane*. The equivalence of RNA loading among *lanes* was demonstrated by ethidium bromide staining of rRNA. *NT* indicates RNA from the non-transformed control cell lines. + and − indicate presence or absence of sucrose in medium. *A*, suspension cells of transgenic rice (cv. Tainung 62) cell lines C7 and C11. *B*, suspension cells of transgenic rice (cv. Tainan 5) cell line C51, tobacco cell line TO1, and potato cell line P1.

grown with sucrose (Fig. 3*A*, *lanes 1*, *3*, and *5*) but significantly increased after cells were starved of sucrose (Fig. 3*A*, *lanes 2*, *4*, and *6*). Surprisingly, expression of α-amylase genes in the transformed cells under sucrose starvation was significantly lower than that in the non-transformed cells. Our finding that expression of both the GUS gene and the α-amylase genes in the transformed cells was similarly regulated by sucrose in the medium indicates that the 1.2-kb 5′ region of *αAmy8* contains all the cis-elements required for responsiveness to sugar suppression. In addition, the rice α-amylase promoter is functional and regulated by sugar nutrients not only in rice cells but also in tobacco and potato cells.

Expression of αAmy8/GUS in Transgenic Cell Lines at Protein Level—To determine whether the accumulation of GUS mRNA resulted in synthesis of GUS, proteins were extracted from cells grown in sucrose-containing or sucrose-free medium. Accumulation of GUS was determined by Western blot analysis (Fig. 4). No GUS was detected in rice cell lines C7 and C11 grown with sucrose (Fig. 4*A*, *lanes 3* and *5*), but amounts of GUS were significantly increased after cells were starved of sucrose for 48 h (Fig. 4*A*, *lanes 4* and *6*). Because the 1.2-kb 5′ region of *αAmy8* contains a putative signal sequence fused in frame to the GUS coding region (Fig. 1), we also examined whether GUS was present in the culture medium. Total protein in the culture medium was collected, and GUS was detected by Western blot analysis. GUS was detected in the culture medium only when the two cell lines were grown in medium lacking sucrose (Fig. 4*A*, *lanes 11* and *13*) and was undetectable when cells were grown in medium containing sucrose (Fig. 4*A*, *lanes 10* and *12*). Accumulations of endogenous α-amylases in the transformed and non-transformed cells and in their culture media were also detected only when cells were starved of sucrose (Fig. 4*A*, *lanes 2*, *4*, *6*, *9*, *11*, and *13*). Accumulation of α-amylases in the transformed cells and in their culture media under sucrose starvation was also lower compared with the non-transformed cells.

Expression of GUS in another rice variety, in tobacco, and in potato was also determined. Levels of GUS were low in rice cell lines C51 and C52 when cells were grown with sucrose (Fig. 4*B*, *lanes 3* and *5*) but increased 5–7-fold when cells were grown without sucrose (Fig. 4*B*, *lanes 4* and *6*). Similar patterns of accumulation of GUS in the culture media were observed. Levels of GUS in the sucrose-free medium (Fig. 4*B*, *lanes 11* and *13*) were 15–20 times greater than those in the sucrose-containing medium (Fig. 4*B*, *lanes 10* and *12*). Expression levels of GUS in transgenic tobacco (Fig. 4*C*) and potato (Fig. 4*D*) were similar to that in transgenic rice. The quantity of GUS accu-

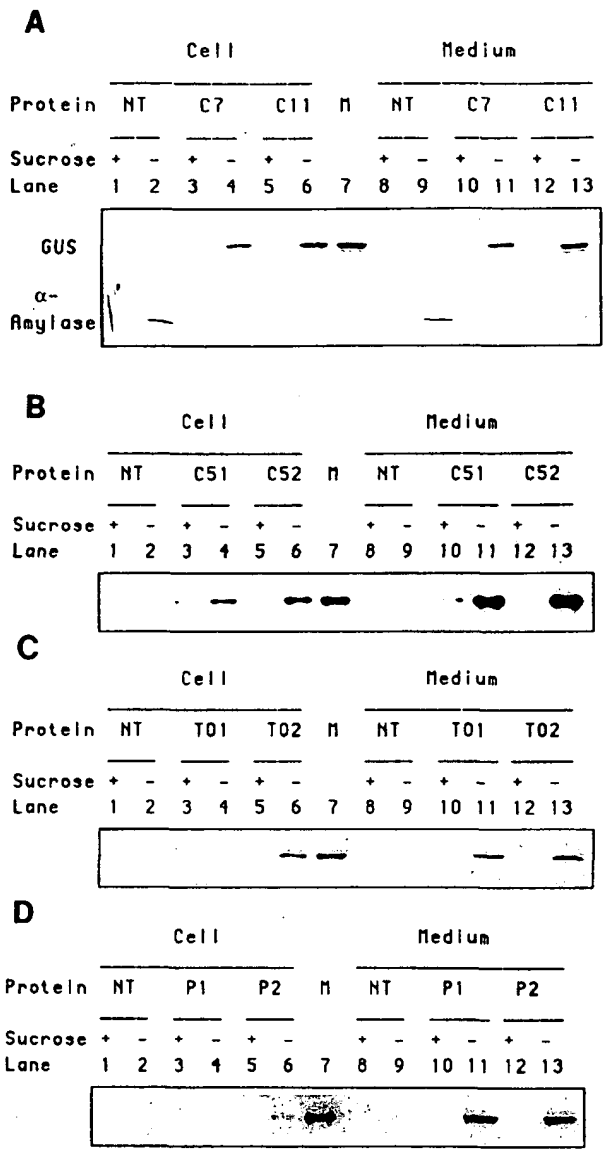

FIG. 4. **Accumulation of GUS in suspension cells and culture medium of transgenic plants.** Suspension cells of transgenic cell lines were grown in sucrose-containing medium for 5 days and transferred to sucrose-containing or sucrose-free medium for 2 days. Proteins were extracted from cells or collected from the culture medium. GUS or α-amylases were detected by Western blot analysis using the GUS or α-amylase antibodies. Twenty μg of total cellular proteins were applied in each of *lanes 1–6*. Five μg of total proteins from medium were applied in each of *lanes 8–13*. *NT* indicates proteins from the non-transformed control cell lines or their culture media. + and – indicate presence or absence of sucrose in the medium. *A*, proteins from two transgenic rice (cv. Tainung 62) cell lines C7 and C11. *Lane 7* (*M*) contains 500 ng of purified *E. coli* GUS protein. *B*, proteins from two transgenic rice (cv. Tainan 5) cell lines C51 and C52. *Lane 7* (*M*) contains 1 μg of purified *E. coli* GUS. *C*, proteins from two transgenic tobacco cell lines TO1 and TO2. *Lane 7* (*M*) contains 400 ng of purified *E. coli* GUS. *D*, proteins from two transgenic potato cell lines P1 and P2. *Lane 7* (*M*) contains 400 ng of purified *E. coli* GUS.

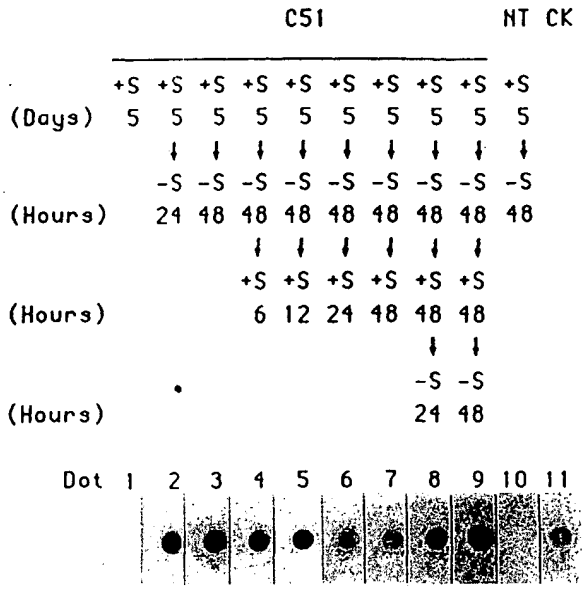

FIG. 5. **Reversibility of expression of GUS in transgenic rice.** Suspension cells of transgenic rice cell line C51 were grown in sucrose-containing medium for 5 days and transferred to sucrose-free medium for 48 h. The starved cells were then transferred to sucrose-containing medium for 48 h and transferred again to sucrose-free medium for 48 h. Suspension cells of the non-transformed cell line were starved in sucrose-free medium for 48 h. Cells were washed in fresh sucrose-free or sucrose-containing medium prior to transfer to the indicated medium. Cells were collected at various times and proteins were extracted. Dot-blot analysis of GUS was performed as described under "Experimental Procedures." Twenty μg of total proteins was applied in each *dot*. *Dot 11* (*CK*) contains 400 ng of purified *E. coli* GUS. *NT* indicates proteins from the non-transformed control cell line. +S and –S indicate medium with and without sucrose, respectively.

mulated in cells or medium under sucrose starvation varied among different transgenic cell lines or plant species. No GUS was detected in cells or culture medium of any of the non-transformed cells (Fig. 4, *A-D*, *lanes 1*, *2*, *8*, and *9*). These results indicate that accumulation of GUS in cells or culture media of transgenic cell lines could be significantly increased by depletion of sucrose in the media. In addition, the putative signal sequence of *αAmy8* is capable of directing translocation of GUS through the secretory pathway of transgenic rice, tobacco, and potato cells.

Reversibility of Expression of αAmy8/GUS in Transgenic Rice—To analyze this phenomenon, suspension cells of transgenic rice cell line C51 were alternately grown in sucrose-free and sucrose-containing medium for various times. Cells were collected at various time points, total cellular proteins were extracted, and GUS was detected by dot-blot analysis (Fig. 5). GUS was undetectable in cells grown in sucrose-containing medium for 5 days (Fig. 5, *dot 1*) but was greatly increased after 24 and 48 h of sucrose starvation (Fig. 5, *dots 2* and *3*). When the starved cells were returned to sucrose-containing medium, the level of GUS gradually decreased during 48 h of incubation (Fig. 5, *dots 4–7*). GUS level increased again after cells were transferred back into sucrose-free medium for 24 and 48 h (Fig. 5, *dots 8* and *9*). No GUS was detected in the non-transformed control cells even after 48 h of starvation (Fig. 5, *dot 10*). This experiment demonstrates that the induction or suppression of *αAmy8*/GUS expression in transgenic rice is reversible and mediated by the presence or absence of sucrose in the medium.

ER Targeting and Glycosylation of GUS in Transgenic Plants—Two potential sites for *N*-linked glycosylation (Kornfeld and Kornfeld, 1985) are present in the deduced amino acid sequence of *Escherichia coli* GUS (Jefferson *et al.*, 1986). To determine whether the signal sequence of *αAmy8* could target GUS to the ER and within which the *N*-linked glycosylation of GUS could occur, proteins were extracted from suspension cells of the transgenic cell lines or collected from their culture media and subjected to Western blot analysis (Fig. 6*A*). GUS present in the cell extract of transgenic rice, tobacco, and potato (Fig. 6*A*, *lanes 1*, *3*, and *5*) had a molecular mass of 70 kDa, which

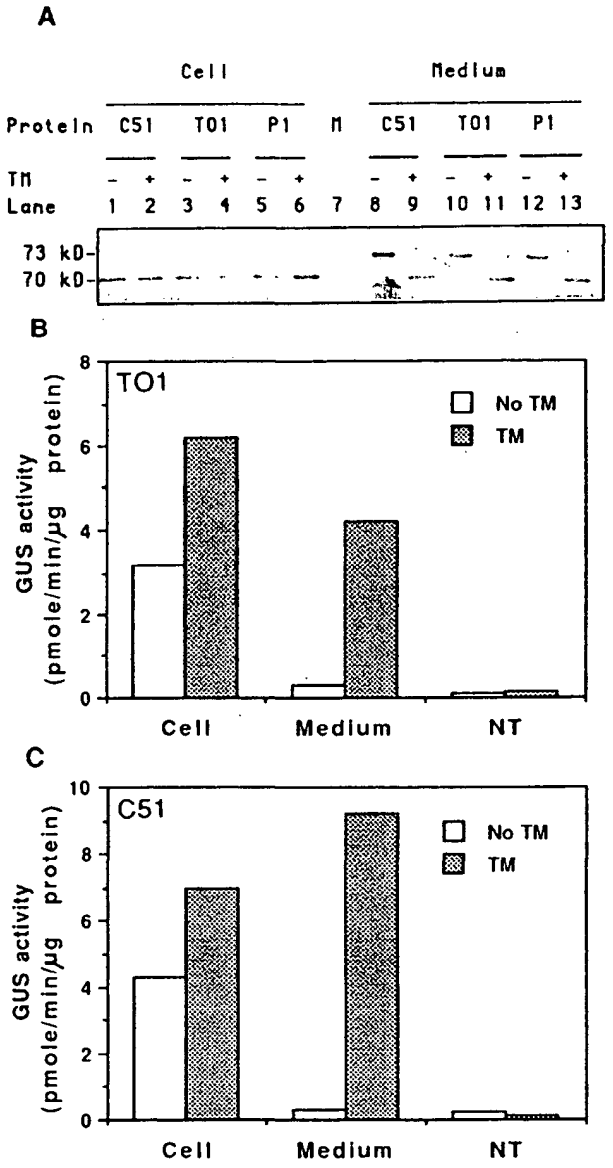

FIG. 6. **Analysis of *αAmy8*/GUS fusion protein in suspension cells treated with TM.** Suspension cells of transgenic rice (C51), tobacco (TO1), and potato (P1) were grown in sucrose-free medium containing 10 μg/ml TM for 24 h. The control cells were grown in sucrose-free medium containing an equivalent volume of the solvent used for dissolving TM. Proteins were extracted from cells or collected from the culture medium. *A*, Western blot analysis of GUS using the GUS antibodies. Twenty μg of total cellular proteins was loaded in each of *lanes 1–6*. Five μg of total proteins from medium was loaded in each of *lanes 8–13*. *Lane 7* (*M*) contains 200 ng of purified *E. coli* GUS. + and − indicate presence or absence of TM in medium. *B* and *C*, assay of GUS activities in tobacco (TO1) and rice (C51), respectively. *NT*, GUS activities in the non-transformed control cells.

was identical to that of the purified native GUS from *E. coli* (Fig. 6*A*, *lane 7*). On the other hand, GUS collected from the culture media of these cells (Fig. 6*A*, *lanes 8*, *10*, and *12*) had a molecular mass of 73 kDa. The increase in molecular mass of the secreted GUS was probably due to the addition of oligosaccharides by oligosaccharide transferase, which is found on the lumenal side of the ER (Hirschberg and Snider, 1987). Tunicamycin (TM) specifically inhibits the transfer of oligosaccharide side chain to the NX(S/T) residues of proteins by oligosaccharide transferase in the lumen of the ER (Elbein, 1987). Treatment of cells with TM should block the glycosylation of GUS and consequently decrease the molecular mass of the secreted GUS. This possibility was examined by treating the suspension cells of the various transgenic cell lines with 10 μg/ml TM for 24 h. Proteins were then extracted from the treated cells or collected from their culture media and analyzed. The molecular mass of GUS remained 70 kDa in the cell extracts of rice, tobacco, and potato (Fig. 6*A*, *lanes 2*, *4*, and *6*), whereas the molecular mass of GUS collected from the culture media was reduced from 73 to 70 kDa following TM treatment (Fig. 6*A*, *lanes 9*, *11*, and *13*). These results indicate that GUS fused to the signal sequence of *αAmy8* could be transported to the ER, glycosylated in the lumen of the ER, and secreted from the cells.

Glycosylation of GUS inhibits the enzyme's catalytic activity, and TM treatment allows the synthesis of active GUS in transgenic tobacco (Iturriaga *et al.*, 1989; Pan *et al.*, 1992). When we determined the GUS activity in cells or their culture media, GUS activity in the tobacco suspension cells (Fig. 6*B*) was 11-fold greater than in the medium and increased only 2-fold following TM treatment. In contrast, GUS activity in the culture medium of tobacco cells was originally very low and increased 14-fold following TM treatment (Fig. 6*B*). The effect of TM treatment was even more striking for rice suspension cells. The GUS activity in rice suspension cells was 14-fold greater than in the medium and increased only 1.6-fold following TM treatment, whereas the activity in the culture medium was originally very low and increased 30-fold following TM treatment (Fig. 6*C*). The GUS activity remained at background level in the non-transformed cells or their culture medium. These experiments suggest that the low activity of GUS in the culture medium of untreated tobacco or rice cells is due to glycosylation of the GUS protein and that the secreted GUS is active when it is not glycosylated, such as occurs in TM-treated cells.

DISCUSSION

Previously, we have shown that the metabolic regulation of α-amylase gene expression involves a transcriptional control mechanism (Sheu *et al.*, 1994). Here we show that the 1.2-kb 5′ region of a rice α-amylase gene, *αAmy8*, directs the expression of GUS in transgenic rice; the expression is repressed by the presence of sucrose and induced by its absence. The experiments provide evidence that this 1.2-kb DNA fragment possesses the necessary cis-regulatory sequences for conferring metabolic regulation of its downstream gene. Expression of the *αAmy8*/GUS chimeric gene was regulated similarly in transgenic potato and tobacco, indicating that the regulatory sequences within the rice α-amylase promoter could be recognized by these two plant species. This suggests that the pathway for transducing signals of sugar starvation and the regulatory mechanism of sugar suppression on the α-amylase promoter are conserved and retain common features between monocots and dicots during evolutionary divergence of the two angiosperm lines.

The inducibility of the α-amylase promoter in cultured cells by sugar starvation makes it potentially useful for the design of inducible gene expression systems for transfected genes. An ideal promoter for conditional expression of genes in plants should fulfill several criteria: 1) a low level of expression under uninduced condition, 2) a high level of expression under induced condition, 3) induction that is readily reversible, and 4) proper regulation in a number of heterologous systems. Expression of *αAmy8*/GUS was low when the transgenic cells were grown in medium containing sucrose but became high after cells were incubated in medium lacking sucrose (Figs. 3 and 4). Induction or suppression of *αAmy8*/GUS expression could be readily reversed by the manipulation of sugar level in medium (Fig. 5), which is similar to findings for α-amylases (Yu *et al.*,

1991). GUS is very stable in cells with a half-life in living mesophyll protoplasts of approximately 50 h (Jefferson *et al.*, 1987). It may explain why GUS was still detectable 48 h after cells were shifted to sucrose-containing medium (Fig. 5, *dot 7*) while accumulation of GUS mRNA had become undetectable under the same condition (Fig. 3*B*, *lane 3*). In addition, the rice α-amylase promoter can be expressed and properly regulated in rice, tobacco, potato, and probably in most plant species. Therefore, the rice α-amylase promoter appears to satisfy the above criteria and is suitable for establishing an inducible expression system in tissue cultures. Such an expression system offers opportunities not only to study the physiological functions of certain gene products but also to control important steps in plant metabolism and development.

The 1.2-kb 5′ region of *αAmy8* contains a putative 25-amino acid signal sequence that facilitates extracellular targeting of GUS in transgenic rice, tobacco, or potato cells. Evidence for translocation of GUS to the cell membrane via the ER of the plant cells is based on our observation that cells transformed with the *αAmy8*/GUS chimeric gene secreted a 73-kDa GUS molecule into the culture medium (Fig. 6*A*), and activity of the secreted GUS was significantly reduced (Fig. 6, *B* and *C*). Treatment of these cells with TM resulted in a decrease of molecular mass (70 kDa) (Fig. 6*A*) and an increase in activity of GUS secreted into the culture medium (Fig. 6, *B* and *C*), suggesting that GUS is transported to the ER, glycosylated in the lumen of the ER, and secreted from the cells. Therefore, the signal sequence of *αAmy8* apparently allows secretion of the expressed passenger proteins that need to be modified and assembled into active molecules in the ER. The slightly increased GUS activity in the suspension cells of tobacco or rice resulting from TM treatment (Fig. 6, *B* and *C*) suggests that some intracellular GUS was glycosylated but remained undetectable by Western blot analysis (Fig. 6*A*). Another possibility is that TM treatment may somehow stabilize the intracellular GUS.

The observation that active GUS is formed following TM treatment means that GUS is assembled into an active tetramer in the lumen of the ER. This is significant because several desirable proteins need to be post-translationally modified and assembled in the ER to create active molecules. Our observations indicate that the signal peptide of *αAmy8* will be useful for obtaining active translocated proteins. To evaluate the potential as a secreted protein expression system, the efficiency of GUS secretion from the cultured cells of different plant species was determined and compared. Total proteins were collected from the culture media and subjected to Western blot analysis (data not shown) in which various amounts of the purified *E. coli* GUS were used as the standard for estimating the yield of GUS secreted by the various transgenic cell lines. The percentage of GUS in total proteins collected from the culture media ranged from 10 to 40% (Table I). These results indicate that the promoter and signal sequence of *αAmy8* can be used for expression and secretion of foreign proteins in plant cell cultures derived from different plant species. However, the yield of GUS varied between different transgenic cell lines of the same plant species as well as among different plant species. The comparative yields in these transgenic cell lines were rice (Tainan 5) > rice (Tainung 62) > tobacco > potato. Variations in the level of GUS expression may result from position and/or copy number effects of introduced genes in different transgenic cell lines or species. Copy number effects can be excluded because except for one transgenic tobacco cell line (TO2), which has two copies of the GUS gene, each of the other transgenic cell lines has only one copy (Fig. 2). We are now performing protein yield analysis on more transgenic cell lines of various plant species to determine whether the expression efficiency of recombinant proteins varies with different plant species or varieties.

TABLE I
Yield of GUS produced by suspension cells of different transgenic plant species

Transgenic cell lines[a]	Yield		Total protein[d]
	μg/g[b]	μg/liter[c]	
			%
Rice			
C7	135	1080	10
C11	156	1248	10
C51	370	2960	40
C52	303	2424	40
Tobacco			
TO1	87	696	25
TO2	48	384	15
Potato			
P1	29	232	15
P2	31	248	20

[a] Suspension cells were grown in sucrose-free medium for 2 days. Proteins were collected from the culture medium, fractionated by SDS-polyacrylamide gel electrophoresis, and analyzed by Western blot analysis using the GUS antibodies. GUS was quantified by comparison with a known amount of purified *E. coli* GUS loaded on the same gel.
[b] Total amount of GUS collected from culture medium/fresh weight of cells incubated.
[c] Yield (μg/g) × 0.4 g/50 ml (or 8 g/liter).
[d] Total amount of GUS collected from culture medium/total amount of proteins collected from culture medium.

Our studies suggest that the *αAmy8* promoter/signal sequence/plant cell culture system constitutes a superb choice for expression of secreted recombinant proteins. Advantages of this plant protein expression system are as follows. 1) The culture medium is less expensive than the culture media for other gene expression systems. 2) The culture medium contains no exogenous added protein. 3) The system is capable of performing post-translational modifications of eukaryotic proteins. 4) Purification of the recombinant proteins is simplified due to secretion of protein into the culture medium. 5) Transcription of the α-amylase promoter-driven foreign genes is well regulated by sugar nutrients in the medium, which is important when continuous overexpression of the desired product is deleterious. The dual control characteristics of this system also offer an attractive approach to fermentation in which the host strain is first cultured in the presence of sugar nutrients to maximize biomass effectively and is then cultured under sugar-depleted conditions to elicit production of the desired protein.

The preliminary yield studies indicate that the yield of GUS from transgenic rice cell line C51 was 370 μg of GUS/g (Table I) (or 2960 μg of GUS/liter) of cells/48 h starvation period, which was produced by only 0.4 g of cells incubated in 50 ml of sucrose-free MS medium for 48 h. The ratio of cell mass to medium could presumably be greatly increased since no growth of these cells is required. Thus, if more cells were incubated in the sucrose-free MS medium and assuming that the yield of GUS increases with cell mass, the estimated yield of GUS would be significantly increased. Experiments are currently under way to optimize the conditions for rice cell fermentation.

In conclusion, the experiments described here demonstrate that expression of the rice α-amylase promoter/GUS reporter gene in different transgenic plant species can be controlled by sugar level in the medium, which allows the functional analysis of the promoter sequence important for its response to sugar regulation. The α-amylase promoter can be engineered into an inducible expression cassette for basic studies of the cell biological effects of a given gene product by controlling the levels of expression of this transfected gene. In conjunction with the

signal sequence of α-amylase, this expression cassette can also be used for production of secreted recombinant proteins. This expression system offers novel approaches for basic research in plant biology as well as for large scale production of genetically engineered proteins.

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High Level of Expression of Recombinant Human Granulocyte-Macrophage Colony Stimulating Factor in Transgenic Rice Cell Suspension Culture

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Abstract: Recombinant human granulocyte-macrophage colony stimulating factor (hGM-CSF) has been previously produced in tobacco cell suspension cultures. However, the amount of hGM-CSF accumulated in the culture medium dropped quickly from its maximum of 150 μg/L at 5 d after incubation. To overcome this problem, we sought an expression system in which heterologous gene expression could be induced at high levels. We selected a rice amylase expression system in which the promoter Ramy3D is induced to express recombinant protein by sucrose starvation. This induction system was found to give good yield of recombinant hGM-CSF in transgenic rice cell suspension culture and protease activity of this culture medium was low compared to that of tobacco culture system. © 2003 Wiley Periodicals, Inc. *Biotechnol Bioeng* **82**: 778–783, 2003.

Keywords: Ramy3D; recombinant human GM-CSF; transgenic rice cell suspension culture

INTRODUCTION

Many different expression systems, including microbial, insect, mammalian, and plant cell systems, have been used for production of heterologous enzymes, cytokines, antibodies, and other recombinant proteins. Each of these systems has both advantages and disadvantages for the production of recombinant proteins. The rapid accumulation of knowledge about the mechanisms of plant gene regulation has enabled development of economically competitive plant cell systems for production of bioactive recombinant proteins of commercial value (Daniell et al., 2001). Although possible product contamination by mycotoxins and other secondary metabolites, is important consideration in the use of plant cell culture systems to produce recombinant proteins, these systems offer some advantages over animal cell culture systems (Doran, 2000). First, recombinant proteins produced by transformed plant cells are more likely to be safe for human consumption, because plant pathogens, such as fungi and viruses, are easily monitored and usually not pathogenic to humans. Second, sexual crossing can generate multiple transgenic plants. Third, the ease of downstream purification and the low cost of plant culture media, which contain inexpensive major components such sucrose and salt and no macromolecules, make plant cell culture an economically attractive alternative. Therefore, plant cell culture systems may be the most favorable means of producing small-to-medium quantities of high-priced, high-purity, specialty recombinant proteins, despite the fact that these systems are relatively new. (Doran, 2000).

Human granulocyte-macrophage colony stimulating factor (hGM-CSF) was one of the first of a large number of cytokines to be purified and cloned (Lee et al., 1985). Mature hGM-CSF, which contains 127 amino acid residues, results from the cleavage of a hydrophobic 25-amino-acid leader sequence. This cytokine has increasing clinical applications in the treatment of neutropenia and aplastic anemia, and has greatly reduced the infection risk associated with bone marrow transplantation by accelerating the response of neutrophils (Rasko and Gough, 1994).

We previously produced hGM-CSF using transformed tobacco cells in suspension cultures, and demonstrated that it was secreted into the culture medium in a bioactive form (Lee et al., 2002). The amount of hGM-CSF present in the culture medium increased over time to a maximum of 150 μg/L at day 5 after incubation and dropped quickly, concomitant with high protease activity in the culture medium. To overcome this problem, we used the rice amylase expression system Ramy3D (Toyofuku et al., 1998) to manufacture recombinant hGM-CSF in rice cell suspension culture, and found that the yield of hGM-CSF was significantly

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Exhibit C

higher than that obtained in our previous transgenic tobacco expression system.

MATERIALS AND METHODS

Construction of the Expression Vector

Total poly-$(A)^+$ mRNA was isolated from peripheral blood mononuclear cells stimulated by PHA and human interleukin-12. RT-PCR with the primers 5′-TCT GCG CAC CCG CCC GCT CGC-3′ and 5′-TTG GTA CCA TCT GGC CGG TCT CA-3′ was used to synthesize the cDNA for hGM-CSF. The resulting 411-bp PCR fragment contained the open reading frame for hGM-CSF lacking its signal peptide (Lee et al., 1985). This fragment was cloned into the pGEM-T vector (Promega, WI) to generate pMYN24. The DNA fragment containing the Ramy3D promoter and signal peptide coding sequence (Toyofuku et al., 1998) was amplified with primers 5′-GAG CAT GCA CCA CCT GTG CTA GCT ACT CCA CTG-3′ and 5′-AAC TGC AGG CTT GAC CCG AGT TAC AGG TC-3′. This PCR product was digested with *Sph*I and *Pst*I and subcloned into the pUC18 multiple cloning site. The resulting plasmid, pMYN24, was digested with *Fok*I and *Kpn*I to excise the gene for hGM-CSF, which was sub-cloned into the *Pst*I (blunted with T4 DNA polymerase)–*Kpn*I site of pMYN27. The resulting plasmid, pMYN32, was digested with *Hin*dIII and *Kpn*I, and a DNA fragment containing hGM-CSF with the Ramy3D promoter and signal peptide coding sequence was inserted into the binary vector (Fig. 1).

Transformation and Screening of Transgenic Rice Calli

Rice calli were prepared and transformed by the method of particle bombardment-mediated transformation (Chen et al., 1998). After bombardment, explants were transferred to selection medium N6 (Chu et al., 1975) supplemented with 2,4-dichlorophenoxyacetic acid (2 mg/L), sucrose (30 g/L), proline (0.5 g/L), glutamine (0.5 g/L), casein enzymatic hydrolysate (0.3 g/L), and hygromycin B (35 mg/L) every 2–3 weeks. Calli resistant to hygromycin B were grown in N6 media minus sucrose (N6S-S) for 3 days and then analyzed. The amount of recombinant hGM-CSF in transgenic calli was measured by an hGM-CSF-specific ELISA kit (Endogene, Woburn, MA) according to the procedure provided by the manufacturer.

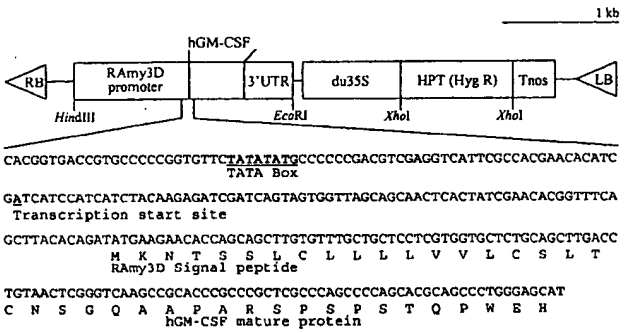

Figure 1. Schematic diagram of the gene construct utilized in this study. Transferred DNA (T-DNA) of the final plasmid is shown. RB, T-DNA right border; 3′UTR, 3′ untranslated region of rice α-amylase gene; du35S, CaMV35S promoter with an enhancer region; HPT, hygromycin phosphotransferase; Tnos, terminator of nopaline synthase; LB, T-DNA left border.

Establishment of Cell Suspension Cultures and Assay for Protease Activity

Calli that were found to express high levels of hGM-CSF were used to establish cell suspension cultures. These cultures were grown at 25°C in a 50-mL volume of AA+S medium [AA medium (Thompson et al., 1986) containing 2,4-dichlorophenoxyacetic acid (2 mg/L), kinetin (0.02 mg/L), and 3% sucrose] in the dark at 110 rpm in a shaking incubator. A 10-mL inoculum was passaged every 9 days for subculturing. To induce hGM-CSF expression, the AA+S medium was removed from the cell suspension by aspirating, and the cells were transferred to fresh AA-S medium at 10% (weight of wet cells/volume of AA-S medium) density. To check protease activity of culture medium in different conditions, the suspended cells were collected from AA+S medium by aspiration, and 2 g of cells was inoculated into 50 mL of fresh AA+S and AA-S medium, respectively. Protease activity in the culture medium was measured according to the method of Battaglino et al. (1991) at different times.

Northern and Western Blot Analyses

For Northern blot analysis, total RNA was isolated from suspension cells grown in AA+S and AA-S liquid media using the RNeasy plant total RNA extraction kit (Qiagen, CA). Northern blot analysis was carried out according to the procedure of Sambrook et al. (1989). An *Eco*RI fragment from pMYN24 was labeled by random-priming with α-^{32}P-ATP and used as a probe. For Western blot analysis, samples were electroblotted onto PVDF membranes after SDS-PAGE (Towbin et al., 1979). Rat monoclonal anti-hGM-CSF antibody (Pharmingen Inc., CA) at 1:200 and HRP-conjugated goat anti-rat IgG (Sigma, MO) at 1:7,000 were used as primary and secondary antibodies, respectively.

Quantitative and Functional Assays for Recombinant hGM-CSF

After production of recombinant hGM-CSF was induced by incubation of transgenic rice cells in AA-S liquid medium, the suspension culture was centrifuged at 200*g* for 3 min. A 1-mL sample of the resulting culture supernatant was dialyzed against phosphate-buffered saline overnight at 4°C and then used for analysis. The concentration of recombi-

nant hGM-CSF was determined using an hGM-CSF-specific ELISA kit (Endogene, Woburn, MA) according to the manufacturer's instructions.

To determine the biological activity of the recombinant hGM-CSF, the amount of culture supernatant required to support growth of hGM-CSF-dependent TF-1 cells was measured (Kitamura et al., 1989). Briefly, a 100-μL aliquot of growth-factor-starved cells suspended in RPMI medium supplemented with 10% FBS (HyClone Laboratories Inc., UT) at 1×10^6 cells/mL was added to each well of a microtiter plate containing test samples of hGM-CSF culture supernatant. The plates were incubated for 48 h, and then 1 μCi of [methyl-^{3}H]thymidine (Amersham Life Science, NJ) was added to each well. The plates were incubated for an additional 16 h. The cells were then harvested using an Inotech cell harvester (Switzerland), and the tritium content was measured by liquid scintillation counting. Recombinant *E. coli*-derived hGM-CSF was purchased from Endogene (Boston, MA) and used as a positive control for activity.

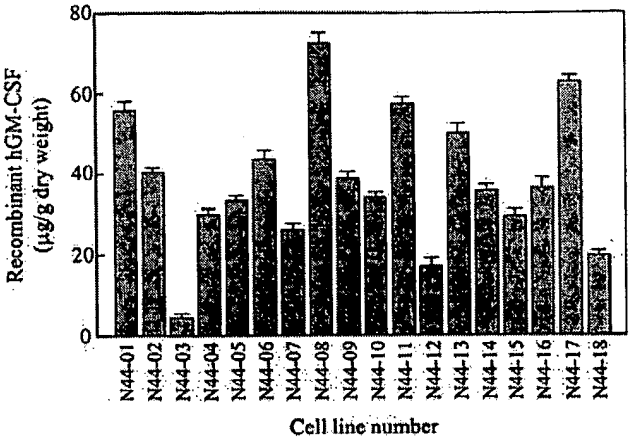

Figure 2. Screening of transformed callus lines expressing high levels of hGM-CSF. The amount of hGM-CSF was determined by ELISA as described in Materials and Methods.

RESULTS

Vector Construction and Transformation

The structure of the hGM-CSF expression vector pMYN44, which contains the hygromycin phosphotransferase (HPT) gene as a selection marker, is shown in Figure 1. Since expression of the hGM-CSF gene on this vector is controlled by the Ramy3D promoter, expression can be induced by removing the sucrose from the culture. Rice calli were transformed with the pMYN44 plasmid by particle bombardment. The presence of the hGM-CSF gene in calli was detected by PCR with primers designed to amplify the hGM-CSF gene (data not shown). Eighteen callus lines were selected for further analysis and named N44-01 to N44-18.

Northern and Western Analysis for Production of hGM-CSF RNA and Protein

To confirm expression of hGM-CSF, Northern and Western blot analyses were conducted. Northern blot analyses of 18 callus lines revealed various levels of hGM-CSF mRNA expression (data not shown). As shown in Figure 2, hGM-CSF levels in the selected callus lines, which were measured at day 3 after transfer to sucrose-free medium, varied widely. Line N44-3 expressed recombinant hGM-CSF at less than 4 mg/g cells (dry weight), whereas some other lines expressed hGM-CSF at over 40 mg/g cells. Line N44-8 produced the highest level of recombinant hGM-CSF (73 mg/g cells) and was selected for further analysis.

As shown in Figure 3A, levels of hGM-CSF mRNA in N44-8 increased until day 7 after induction and then decreased slowly. A little hGM-CSF mRNA was expressed by day 19 after induction or in the growth phase. Levels of recombinant hGM-CSF protein accumulated in culture medium were determined by Western blot analysis (Fig. 3B). In Figure 3B-1, there were faint bands in lane 1 and 2, which corresponded to day 9 in growth phase and day 1 in production phase, respectively. As shown in Figure 5A, there was little sucrose in culture medium after day 7 in growth phase due to consumption by rice cells, which induced the Ramy3D expression system. The non-glycosylated *E. coli*-derived recombinant hGM-CSF appeared as a band at ~14 kDa on the Western blot (Fig. 3B-2). Culture medium from the transgenic rice cells produced two distinct bands of ~30 kDa that may reflect micro-heterogeneity caused by variable glycosylation. Human GM-CSF has two sites for N-glycosylation. Two sites for N-glycosylation which is a major glycosylation can cause big molecular weight shift. Amino acid sequencing of N-terminal and C-terminal confirmed correct protein.

The amount of hGM-CSF in the culture medium increased after induction to a maximum at day 11. Any fragment with lower molecular weight was not detected.

Protease Activity in the Culture Medium

The levels of protease activity in culture media obtained from transgenic plant cells grown in suspension are shown in Figure 4. The protease activity levels in culture media derived from transgenic tobacco cells and from growth and production phase transgenic rice cells were measured. For the transgenic tobacco cell culture, protease activity in the medium increased linearly from the first day of culture. A similar result was found for the growth phase of the rice cell culture, although the protease activity level was about one-half of the level displayed by the tobacco culture medium. In contrast, there was little protease activity in the production-phase culture medium, namely, the sucrose-free medium. The low level of protease activity observed in the production phase together with the strong induction by sucrose starvation was thought to be one of the factors to

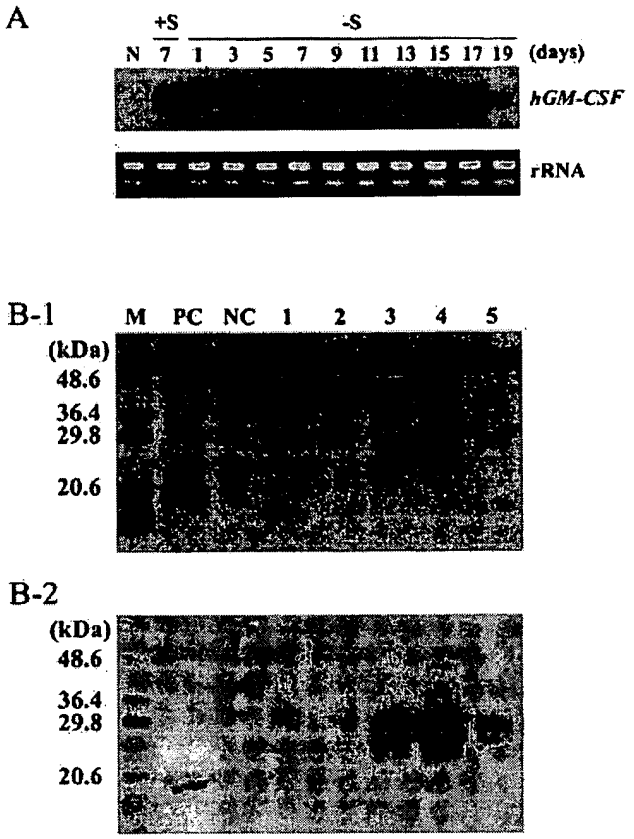

Figure 3. (A) Northern blot analysis to determine the temporal expression pattern of the hGM-CSF gene during plant cell suspension culture. N is non-transformed rice cell culture as a negative control. +S and −S indicate media with and without sucrose, respectively. Loading standards are indicated by ethidium bromide-stained rRNA in the lower panel. (B) SDS-PAGE (**B-1**) and Western blot analysis (**B-2**) of hGM-CSF in culture medium. Lanes M, PC, and NC denote prestained molecular weight standards, positive control hGM-CSF derived from *E. coli*, and negative control culture medium from suspension cells transformed with the parent vector only, respectively. Lane 1, total secreted protein at day 9 in the growth phase; lanes 2, 3, 4, and 5, total secreted protein at days 1, 5, 11, and 17 in the production phase, respectively.

increase the amount of hGM-CSF in the medium 1,000-fold over that obtained with our previous system, in which expression in transgenic tobacco cells was under control of the cauliflower mosaic virus (CaMV) 35S promoter.

Quantitative and Functional Assays for Recombinant hGM-CSF

The amount of hGM-CSF in culture medium was determined by ELISA (Fig. 5A). The level of recombinant protein increased sharply in the production phase to a maximum of 129 mg/L at day 13. After day 13, the amount of recombinant hGM-CSF in the culture medium dropped quickly, most likely due to the decrease in transcription observed after day 7 (Fig. 3A) together with the increase in protease activity (Fig. 4). Recombinant hGM-CSF comprised about 25% of the total secreted protein, although the

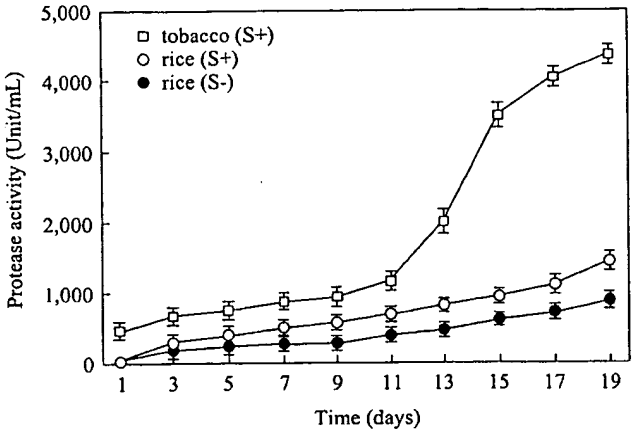

Figure 4. Protease activity in culture medium obtained from suspension cell cultures. Tobacco (S+): culture medium from tobacco cells in suspension in the presence of sucrose. Rice (S+): culture medium from growth-phase rice cells in suspension in the presence of sucrose. Rice (S−): culture medium from production-phase rice cells in suspension in the absence of sucrose.

total amount of secreted protein varied greatly during culture (Fig. 5B).

The biological activity of the recombinant hGM-CSF from rice cell suspension cultures was measured, with *E. coli*-derived hGM-CSF used as a positive control. The culture medium from rice cells transformed with the parent vector only was used as a negative control, and, as expected, it did not support the growth of hGM-CSF-dependent TF-1 cells. However, the recombinant hGM-CSF produced from the N44-8 cell line effectively supported the proliferation of TF-1 cells. The biological activity of the recombinant hGM-CSF tested here matched completely with the data of ELISA, which demonstrated that it was correctly folded and functional. The specific activity of recombinant hGM-CSF in the N44-8 cell suspension culture, as estimated by the degree of [methyl-^{3}H]thymidine uptake per ng of recombinant hGM-CSF, was similar to that of the commercial *E. coli*-derived hGM-CSF.

DISCUSSION

Recombinant murine (Lee et al., 1997) and human GM-CSFs (James et al., 2000; Lee et al., 2002) have previously been expressed and secreted into culture media in a biologically active form. However, hGM-CSF comprised only ~0.5% of the total secreted protein, and it accumulated in the culture media to only 150–250 μg/L (James et al., 2000). This low productivity is an example of the major bottleneck in production of recombinant proteins using plant cell culture. In addition, the small fraction of total protein that is comprised of target protein (0.5% in case of hGM-CSF) effectively negates the ease of downstream purification that is one of the major advantages of a plant cell culture system.

As shown in our previous report (Lee et al., 2002) about

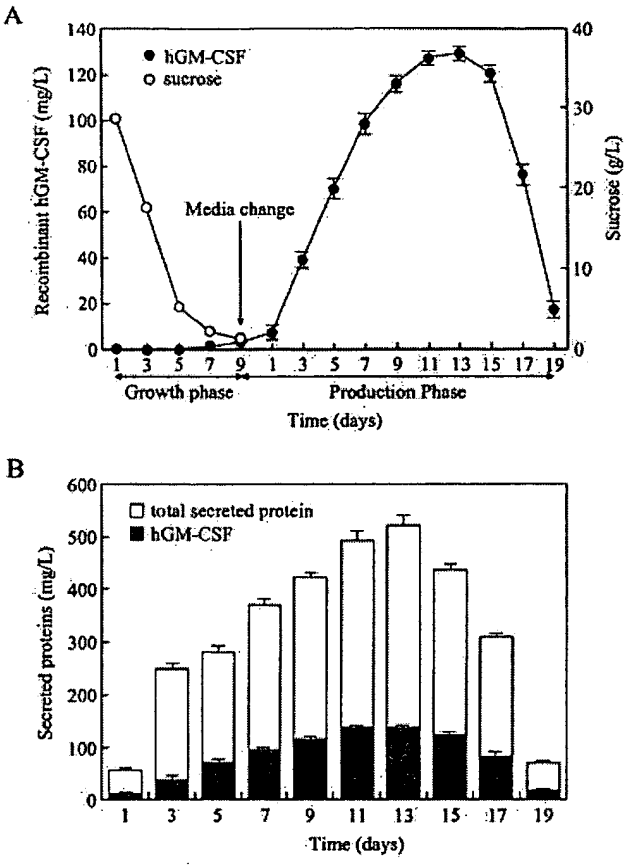

Figure 5. Time course of hGM-CSF (**A**) and total secreted protein (**B**) accumulated in culture medium of suspension cultures as determined by ELISA and Bradford assay, respectively. Rice cells in suspension culture were propagated for 9 days in AA+S media, transferred to AA-S media, and cultured for the lengths of time (days) indicated.

tobacco cell culture, the rapid decrease in the amount of recombinant protein in the culture medium after day 5 was observed. The loss of heterologous proteins during plant cell culture has been reported previously (LaCount et al., 1997; Sharp and Doran, 1999; Terashima et al., 1999; Wongsamuth and Doran, 1997). Indeed, degradation of heterologous proteins after successful synthesis and assembly has been proposed to be at least as important as protein synthesis in determining recombinant protein production levels in plant cell culture.

Hence, we attempted to find culture conditions under which gene expression is strongly induced. As a result, we selected a rice amylase expression system in which the Ramy3D promoter controls expression. When rice callus tissue from the scutellum is placed into suspension culture, the Ramy3D isozyme is one of the most abundant proteins secreted into culture medium upon sucrose starvation (Huang et al., 1993). The Ramy3D expression system was used to produce recombinant proteins by transgenic rice cell culture (Huang et al., 2001, 2002; Terashima et al., 1999). In addition, when we measured the level of protease activity in the culture medium upon induction of the Ramy3D promoter by sucrose starvation, we found that the protease activity was very low, especially initially (Fig. 4). The protease activity of the rice cell culture medium in production phase is about half of that observed in growth phase and one-quarter of that observed in transgenic tobacco cell suspension culture, which is thought to be one of the factors contributing to high yield of recombinant hGM-CSF production observed in our experiment.

By exploiting this expression system, we increased the production of hGM-CSF by up to 1,000-fold compared with our previous expression system, which utilized tobacco cell suspension cultures and the CaMV 35S promoter. The amount of recombinant protein in the medium increased until 13 days after induction, after which it dropped quickly. The maximum amount of hGM-CSF obtained in the rice cell culture medium with the Ramy3D expression system was 129 mg/L, which equals or surpasses the amounts reported for insect and mammalian cell expression systems. As shown in Figure 5B, hGM-CSF comprised 25% of the total secreted protein, whereas it comprised only 0.5% of the total protein secreted by a transgenic tobacco cell suspension culture (James et al., 2000). This high-level of expression of hGM-CSF and large portion of total secreted protein will facilitate downstream purification.

Even though there are several advantages to the use of plant cell culture systems, as stated previously, the major bottleneck in commercialization has been low product yield. With the rapid development of plant molecular biology, many mechanisms of plant gene regulation have been elucidated. As demonstrated in this report, the yield of recombinant hGM-CSF from rice cell suspension culture is at least equal to that obtained from an animal cell expression system. Therefore, plant cell culture systems can provide a valuable alternative for the production of recombinant proteins. The Ramy3D expression system combined with the advantages of plant cell culture is certain to benefit industries dependent on expression technology.

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Exhibit D

RESEARCH

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4. K.A. McDonald, "Characterization of Distillation Nonlinearity for Control System Design and Analysis," in the Shell Process Control Workshop, D. Prett and M. Morari Eds, Butterworth, MA (1987).

5. K.A. McDonald, M.J. Cooney and R.S. Criddle, "Manipulative Variable Selection for Stabilizing Control of a Competitive Mixed Culture", Proceedings of the American Control Conference, June 15-18, Atlanta, GA, 2: 1100-1105 (1988).

6. M.J. Cooney and K.A. McDonald, "Steady State and Transient Analysis in Continuous Fermentation", CACHE Anthology of On-Line Computing Applications in the Undergraduate Chemical Engineering Laboratory, CACHE (1988).

7. K.A. McDonald, A. Palazoglu, and B.W. Bequette, "Impact of Model Uncertainty Descriptions for High Purity Distillation Control", AIChE Journal, 34: 1996-2004 (1988).

8. K.A. McDonald, and A. Palazoglu, "A Multiobjective Predictive Control Solution to the Shell Control Problem," in The Second Process Control Workshop: Solutions to the Shell Standard Control Problem, D. Prett, C. Garcia, and B. Ramaker Eds., Butterworth, MA, (1989)

9. K.A. McDonald, and A.P. Jackman, "Bioreactor Studies of Growth and Nutrient Utilization in Alfalfa Suspension Cultures," Plant Cell Reports, 8: 455-458 (1989).

10. G.L. Young and K.A. McDonald, "Effect of Radiation Shield Angle on Temperature and Stress Profiles During Rapid Thermal Annealing," IEEE Transactions on Semiconductor Manufacturing, 3: 176-182 (1990).

11. C.-I. Chen, K.A. McDonald, and L. Bisson, "Oscillatory Behavior of *Saccharomyces cerevisiae* in Continuous Culture I. Effects of pH and Nitrogen Levels," Biotechnology and Bioengineering, 36: 19-27 (1990).

12. C.-I. Chen, and K.A. McDonald, "Oscillatory Behavior of *Saccharomyces cerevisiae* in Continuous Culture II. Analysis of Cell Synchronization and Metabolism," Biotechnology and Bioengineering, 36: 28-38 (1990).

13. K.P. Fruzzetti, A. Palazoglu, J.A. Romagnoli, and K.A. McDonald, "A Robust/Cautious Model Predictive Controller," Journal of Process Control, 1: 187-196 (1991).

14. G.L. Young, K.A. McDonald, A. Palazoglu, and W. Ford, "Thermal Modelling of Bridgman Crystal Growth Using a Boundary Element Approach," Journal of Crystal Growth, 118: 193-203 (1992).

15. G.L. Young and K.A. McDonald, A. Palazoglu and W. Ford, "Boundary Element Solutions for Free Boundary Convection-Diffusion Problems," Journal of Numerical Heat Transfer, Part A-Applications, 21: 299-311 (1992).

16. M.J. Cooney and K.A. McDonald, "Note on the Monod constant for growth for C. utilis on ammonium in continuous and batch culture," Biotechnology Progress, 9: 93-96 (1993).

17. D.A. Desrochers, J.M. Henderson, and K.A. McDonald, "Building the Confidence of Women Engineering Students: A New Course to Increase Understanding of Physical Devices," Proceeding of the GASAT 7 International Conference, Toronto, Canada, July (1993)

18. J.E. Thorup, K.A. McDonald, A.P. Jackman, N. Bhatia, and A.M. Dandekar, "Ribosome Inactivating Protein Production from *Trichosanthes kirilowii* Plant Cell Cultures," Biotechnology Progress, 10: 345-352 (1994).

19. R. Henes, M.M Bland, J. Darby and K. A. McDonald, "Improving the Academic Environment for Women Engineering Students through Faculty Workshops," J. Engr. Education, 84: 59-67 (1995)

20. J.M. Henderson, D.A. Desrochers, K.A. McDonald, and M.M. Bland, "Building the Confidence of Women Engineering Students With a New Course to Increase Understanding of Physical Devices," J. Engr. Education, 83: 337-342 (1994).

21. K.A. McDonald, A.P. Jackman, J.E. Thorup and A.M. Dandekar, "Plant Callus as a Source of Biochemicals", Applied Biochemistry and Biotechnology, 54: 93-108 (1995).

22. N. van Klaveren, A.N. Palazoglu, K.A. McDonald and A.T. Touchstone, "Interactive Computer Simulations for Integrated Learning," Proceedings of the Fourth International Conference on Foundations of Computer Aided Process Design, AIChE Symposium Series, Vol. 91, 344-347 (1995).

23. M.J. Cooney and K.A. McDonald, "Optimal Dynamic Experiments for Bioreactor Model Discrimination," Applied Microbiology and Biotechnology, 43: 826-837 (1995).

24. N. Bhatia, K.A. McDonald, A.P. Jackman and A.M. Dandekar, "A Simplified Procedure for the Purification of Trichosanthin (A Type I Ribosome Inactivating Protein) from *Trichosanthes kirilowii* Root Tubers", Protein Expression and Purification, 7:143-146 (1996).

25. K. McDonald, "Biochemical Engineering", Engineering Horizons, Spring 1996, pg 11 (1996)

26. K. Fruzzetti, A. Palazoglu, and K.A. McDonald, "Nonlinear Model Predictive Control Using Hammerstein Models" Journal of Control 7:31-41 (1997).

27. G. L. Young, K. A. McDonald and A. Palazoglu, "Thermal Stress Analysis of Crystal Growth in a Horizontal Bridgman Furnace" Journal of Crystal Growth, 171: 361-372 (1997)

28. S.D. Archer, K. A. McDonald and A.P. Jackman, "Effect of Light Irradiance on the Production of Sulfolipids from Anabaena 7120 in a Fed-Batch Photobioreactor," Applied Biochemistry and Biotechnology, 67:15-28 (1997)

29. J.C. Wong, K. A. McDonald, A. Palazoglu and T. Wada, "A New Method for Classifying Process Trends Based on Fuzzy Triangular Representation and Hidden Markov Models, IFAC Symposium ADCHEM '97 Proceedings (1997).

30. N.-J. Remi Shih and K. A. McDonald, "Purification and Characterization of Chitinases from Transformed Callus Suspension Cultures of *Trichosanthes kirilowii* Maxim." Journal of Fermentation and Bioengineering, 84: 28-34 (1997)

31. Michael R. Stoner, Christopher A. Humphrey, Danielle J. Coutts, N.-J. Remi Shih, Karen A. McDonald and Alan. P. Jackman, "Kinetics of Growth and Ribsome-Inactivating Protein Production from *Trichosanthes kirilowii* Plant Cell Cultures in a 5 Liter Bioreactor, Biotechnology Progress 13:799-804 (1997).

32. J. C. Wong, K.A. McDonald, A. Palazoglu and T. Wada, "Application of a Fuzzy Triangular Representation and Hidden Markov Models Classification in the Detection of Abnormal Situations in Refining Processes," Control 97 Proceedings, Sydney, Australia, October 1997, pp 566-571 (1997).

33. N.-J. Remi Shih, K. A. McDonald, A. P. Jackman, T. Girbes, R. Iglesias, "Bifunctional Plant Defence Enzymes with Chitinase and Ribosome Inactivating Activities from *Trichsoanthes kirilowii* Cell Cultures", Plant Science 130: 145-150 (1997).

34. N.-J. Remi Shih, K. A. McDonald, A. M. Dandekar, T. Girbes, R. Iglesias, A.P. Jackman, "A Novel Type-1Ribosome-Inactivating Protein Isolated from the Supernatant of Transformed Suspension Cultures of *Trichosanthes kirilowii*", Plant Cell Reports 17:531-537 (1998).

35. N.-J. Remi Shih, K. A. McDonald, T. Girbes, R. Iglesias, A. Kolhoff and A.P. Jackman, "Ribosome-Inactivating Proteins (RIPs) of Wild Oregon Cucumber (*Marah oreganus*) ", Biological Chemistry, 379: 721-725 (1998).

36. J. C. Wong, K. A. McDonald and A. Palazoglu, "Classification of Process Trends Based on Fuzzified Symbolic Representation and Hidden Markov Models", J. Process Control, 8:395-408 (1998).

37. S. Dagdeviren, K. A. McDonald, and A. P. Jackman, "Production of Sulfolipids from Cyanobacteria in Photobioreactors", in BioHydrogen, Edited by O.R. Zaborsky, Plenum Press, New York, NY, pp 459-466 (1998).

38. R. Krishnan, A.P. Jackman, A.M. Dandekar, and K.A. McDonald, "Expression of Trichosanthin in Transgenic Tobacco Plants and Suspension Cultures", Abstracts of Papers American Chemical Society, 217: BIOT Paper 125 (1999).

39. Karen A. McDonald, Michael R. Stoner, N. Remi Shih and Alan P. Jackman, "*Trichosanthes kirilowii* Plant Cell Culture in a 5 Liter Bioreactor" in Plant Cell and Tissue Culture for the Production of Food Ingredients, Edited by Fu, T.-J., Curtis, W.R. and Singh, G., Kluwer Academic/Plenum Publishers, NY (1999).

40. J. C. Wong, K. A. McDonald, and A. Palazoglu, "Classification of Abnormal Plant Operation Using Multiple Process Variable Trends", Journal of Process Control, 11:409-418 (2001).

41. Karen A. McDonald, Alan P. Jackman and Shaelyn Hurst, "Characterization of Plant Suspension Cultures Using the Focused Beam Reflectance Technique", Biotechnology Letters, 23: 317-324 (2001).

42. Melody M. Trexler, Karen A. McDonald and Alan P. Jackman, "Bioreactor Production of Human Alpha-1-Antitrypsin Using Metabolically Regulated Plant Cell Cultures", Biotechnology Progress 18: 501-508 (2002)

43. Rajesh Krishnan, Karen A. McDonald, Abhaya M. Dandekar, Alan P. Jackman and Bryce Falk, "Expression of Recombinant Trichosanthin, a Ribosome-inactivating Protein, in Transgenic Tobacco", Journal of Biotechnology, 97:69-88 (2002).

Invited Lectures/Workshops:

1. "Characterization of Distillation Nonlinearity for Control System Design and Analysis," Shell Process Control Workshop, Houston, TX, December, 1986.

2. "Characterization of Distillation Nonlinearity for Control System Design and Analysis," University of California, Santa Barbara, CA, March, 1987.

3. "Multivariable Model Development for Fermentation Systems", University of Washington, Seattle, WA, February, 1988.

4. "Manipulative Variable Selection for Stabilizing Control of a Competitive Mixed Culture", University of Nevada, Reno, NV, March, 1988.

5. "Multivariable Modelling of Continuous Fermentation", University of California, San Diego, CA, October 1988.

6. "A Multiobjective Predictive Control Approach to the Shell Control Problem," Shell Process Control Workshop, Houston, TX, December, 1988.

7. "Analysis of Cell Synchronization and Metabolism in Oscillatory Continuous Cultures of *Saccharomyces cerevisiae*," University of Colorado, Boulder, CO, Oct 1989.

8. "Looking into the Bioprocess Engineer's Toolbox," Microbiology 291 Seminar, University of California, Davis, CA, January, 1990.

9. "A Robust/Cautious Model Predictive Controller," EECS Robotics and Control Seminar, University of California, Davis, CA, January, 1991.

10. "A Robust/Cautious Model Predictive Controller," Shell Martinez Manufacturing Complex, Martinez, CA, March, 1991.

11. "Modeling of Temperature and Stress Profiles During Rapid Thermal Processing," American Vacuum Society, Northern California Section, CVD/RTP Users Group, Varian Research Center, Palo Alto, CA, July, 1991.

12. "Modeling Oscillatory Behavior in Continuous Yeast Cultures," University of California, Los Angeles, CA, October 1991.

13. "Plant Cell Culture Bioprocessing," NIH Biotechnology Training Retreat, Napa, CA, February 1992.

14. "Predictive Control of Nonlinear Chemical Processes," MAME Dynamics and Control Seminar Series, University of California, Davis, CA, December 1992.

15. "Plant Callus As a Source of Biochemicals", Frontiers in Bioprocessing III, Boulder, CO, September 1993.

16. "Plant Cell Culture Production of Ribosome Inactivating Proteins", Biotechnology Institute and Root Biology Seminars and Chemical Engineering Department, The Pennsylvania State University, November, 1993.

17. "Plant Cell Culture Production of Ribosome Inactivating Proteins," Fall Department Colloquium, Stanford University, Department of Chemical Engineering Colloquim, December, 1993.

18. "Bioreactors for Cell Culture: Design, Selection and Operation," Seminar in Animal Virology, MIC 296, University of California, Davis, CA, May 1994.

19. "Plant Cell Culture Production of Antiviral Proteins: *Engineering and Medicine Working Together*", Society of Women Engineers, Sacramento Chapter Meeting, Davis, CA, May 1995

20. "Ribosome Inactivating Proteins and Their Potential Pharmaceutical and Agricultural Applications," Site Visit of the Biotechnology Industry Organization, Davis, CA, May 1995.

21. "Plant Cell Culture Production of Antiviral Proteins", Research Seminar, Department of Chemical Engineering, University of Queensland, Brisbane, Australia, August 1995

22. "Institutional and Curricular Programs to Improve Retention of Women in Engineering", Seminar sponsored by the Office of Gender Equity, University of Queensland, Brisbane, Australia, August 1995.

23. "Improving the Academic Environment for Women in Engineering", Workshop presented to faculty and staff, University of Queensland, Brisbane, Australia, August 1995.

24. "Engineering 25: A Hands-on Laboratory Course on How Things Work", Workshop presented to faculty and staff, University of Queensland, Brisbane, Australia, August 1995.

25. "Research in a Microbial/Fermentation Biotechnology Laboratory", Current Issues and Developments in the Field of Biotechnology, UC Davis, Davis, CA, November, 1995

26. "Plant Defense Proteins from Plant Cell Culture", Research Seminar, Department of Chemical and Biochemical Engineering, UC Irvine, Irvine, CA, January, 1997.

27. "Innovations in Biochemical Engineering Curriculum", Workshop participant, Biochemical Engineering X, Kananaskis, Alberta, Canada, May, 1997.

28. "Emerging Issues in Bioengineering Education", Panel Participant, AIChE Annual Meeting, Los Angeles, CA, November, 1997.

29. "Plant Defense Proteins: Novel Production Methods and Potential Agricultural Applications ", Calgene, Davis, CA, January, 1998.

30. "Plant Defense Proteins: Potential Applications And Novel Production Methods", Department of Chemical Engineering, Louisiana State University, Baton Rouge, LA, March, 1998.

31. "The Future of the Chemical Engineering Profession: Perspective of an Engineering Educator", NorCal AICHE Meeting, Berkeley, CA, February, 2000.

32. "Optimization of Metabolically Regulated Transgenic Plant Cell Cultures", Department of Chemical Engineering, Washington State University, Pullman, WA, April, 2000.

33. "BioSTAR Partnership", BioSTAR and LSI Research Forum, March, 2000

34. "Trends in Photobioreactor Technology", Workshop on Bioenergy, Photobiology and Biobased Products: Trends and Industrial Development, UC Davis, September, 2000.

35. "Plant Cell Culture Production of Recombinant Proteins", Department of Chemical Engineering, Iowa State University, Ames, IA, March, 2001.

36. "Challenges for Graduate Students and Faculty", Keynote address, Opportunities in Engineering and Science: Building a Model Program for the Advancement of Women, Buehler Alumni Center, UC Davis, Davis, CA, May, 2001.

37. "Plant Cell Bioprocessing", Opportunities in Engineering and Science Faculty Lunch program, UC Davis, Davis, CA, June 2001.

38. "Transgenic Plant Cell Cultures: Current Status and Future Prospects", Amgen, Thousand Oaks, CA, August 2002.

Papers Presented at Technical Meetings (presenter shown in boldface):

1. "Decoupling Dual Composition Controllers 1. Steady State Results," **Karen McDonald** and Thomas McAvoy, Automatic Control Conference, San Francisco, CA, June, 1983.

2. "Optimal Averaging Level Control," **Karen McDonald**, Thomas McAvoy, and Andre Tits, AIChE Annual Meeting, San Francisco, CA, November, 1984.

3. "Application of Dynamic Matrix Control to Nonlinear Distillation Processes," **Karen McDonald** and Thomas McAvoy), AIChE Annual Meeting, Chicago, IL, November, 1985.

4. "Performance Comparison of Methods for On-Line Updating of Process Models for High Purity Distillation Control," **Karen McDonald**, AIChE Spring Meeting, Houston, TX, March, 1987.

5. "Robustness Analysis of High Purity Distillation Control Using Highly Structured, Correlated Model Uncertainty Descriptions," **Karen McDonald** and Ahmet Palazoglu, AIChE Annual Meeting, New York, NY, November, 1987.

6. "Manipulative Variable Selection for Stabilizing Control of a Competitive Mixed Culture", **Karen McDonald**, Michael Cooney and Richard Criddle, American Control Conference, Atlanta, GA, June, 1988.

7. "Effect of pH and Nitrogen Levels on Oscillatory Behavior of *Saccharomyces cerevisiae* in Continuous Cultures", **Karen McDonald**, Ching-I. Chen and Linda Bisson, AIChE Annual Meeting, Washington, D.C., November, 1988.

8. "Bioreactor Studies of Growth and Nutrient Utilization in Plant Cell Suspension Cultures," **Karen McDonald**, Alan Jackman and Mark Johnson, Tissue Culture Association (Calif. Branch) Meeting, Palo Alto, CA, April, 1989.

9. "A Modified Horizontal Bridgman Design for Single Crystal Growth," **Karen McDonald**, Michael Lucas, A. Palazoglu, and William Ford, Eleventh Conference on Crystal Growth, AACG/West, Fallen Leaf Lake, CA, June, 1989.

10. "Analysis of Cell Synchronization and Metabolism in Oscillatory Continuous Cultures of *Saccharomyces cerevisiae*," **Karen McDonald** and Ching-I. Chen, Poster presented at ACS National Meeting, Miami, FL, September 1989.

11. "Multivariable Dynamic Model for Pure Cultures of *E. coli* and *C. utilis*," Karen McDonald and **Michael Cooney**, ACS National Meeting, Miami, FL, September 1989.

12. "Oscillatory Behavior in Continuous Yeast Bioreactors," Karen McDonald and **Ching-I. Chen**, Poster presented at College of Engineering Board of Visitors Meeting, Davis, CA, October 1989.

13. "Design and Analysis of a Modified Horizontal Bridgman Furnace," Karen McDonald, Ahmet Palazoglu, **G. Young**, and William Ford, AIChE Annual Meeting, San Francisco, CA, November 1989.

14. "Robust Multivariable Predictive Control-A Linear Programming Approach," A. Palazoglu, **K. Fruzzetti**, J. Romagnoli, and Karen McDonald), AIChE Annual Meeting, San Francisco, CA, November 1989.

15. "Bioreactor Studies of Alfalfa Plant Suspension Cultures," **Karen McDonald** and Alan Jackman, AIChE Annual Meeting, San Francisco, CA, November 1989.

16. "Population Balance Model for the Oscillatory Behavior in a Continuous Bioreactor System," **Ching-I. Chen** and Karen McDonald, American Control Conference, San Diego, CA, June 1990.

17. "Modelling and Steady State Predictions for a Continuous Competitive Mixed Culture of *E. coli* and *C. utilis* Grown under Nitrogen Limitation", **Karen McDonald** and Michael Cooney, Poster at ACS National Meeting, Washington, DC, August, 1990.

18. "Multivariable Dynamic Model Development," **Michael Cooney** and Karen McDonald, AIChE Annual Meeting, Chicago, IL, November, 1990.

19. "Modeling Bridgman Crystal Growth Using the Boundary Element Technique," **Karen McDonald**, Greg Young, A. Palazoglu and W. Ford, AACG/West Twelfth Conference on Crystal Growth, Fallen Leaf Lake, CA, May 1991.

20. "Identification of Trichosanthin, a Type I Ribosome Inactivating Protein from *Trichosanthes kirilowii* Explant, Callus and Suspension Culture Media," **Annette Hagewiesche**, Karen McDonald and Alan Jackman, Poster presented at the Southern California Biotechnology Symposium, U.C. Irvine, Irvine, CA, July, 1991.

21. "Multivariable Dynamic Model Development for Continuous Cultures," **Michael Cooney** and Karen McDonald, Southern California Biotechnology Symposium, U.C. Irvine, Irvine, CA, July, 1991.

22. "Identification of Trichosanthin in *Trichosanthes kirilowii* Explant, Callus and Suspension Culture Broth," **Karen McDonald**, Annette Hagewiesche and Alan Jackman, AIChE Annual Meeting, Los Angeles, CA, November 1991.

23. "Identification of Trichosanthin, a Type I Ribosome-Inactivating Protein, from *Trichosanthes kirilowii* Explant, Callus and Suspension Culture Broth," Karen McDonald, **Annette Hagewiesche** and Alan Jackman, Biotechnology Training Grant Retreat, Napa, CA, February, 1992.

24. "Studies of Ribosome Inactivating Protein Production from *Trichosanthes kirilowii* Plant Cell Cultures," **Karen McDonald**, John Thorup and Alan Jackman, AIChE Annual Meeting, Miami, FL, November, 1992.

25. "Kinetic Analysis and Modelling of Anabena SP 7120 Growth in a Well-Stirred, Semicontinuous Light-Limited Bioreactor System," **Y. Ko**, Alan. Jackman, Ben McCoy and Karen McDonald, Poster presented at the Second Annual NIH Biotechnology Training Grant Retreat, Napa, CA, February 1993

26. "Cyanobacteria Photoproduction in a Stirred Reactor: Experiments and Modelling of Growth Under Light-Limited Conditions," **Y. Ko**, Ben McCoy, Alan Jackman and Karen McDonald, ACS Spring National Meeting, Denver, CO, March 1993.

27. "Purification of Closely Related Proteins from *Trichosanthes kirilowii* Cultures Using Perfusion Chromatography," **Nishant Bhatia**, Karen McDonald, Alan Jackman and Abhaya Dandekar, ACS Spring National Meeting, San Diego, CA, March 1994.

28. "Nonlinear Model Predictive Control Using Hammerstein Models," **Keith Fruzzetti**, Ahmet Palazoglu and Karen McDonald, AIChE Spring National Meeting, Atlanta, GA, April 1994.

29. "Interactive Computer Simulations for Integrated Learning" Karen McDonald, Nico van Klaveren, Ahmet Palazoglu and Terrell Touchstone, Poster presented at the Foundations of Computer Aided Process Design (FOCAPD'94), Snowmass, CO, July 1994.

30. "Ribosome Inactivating Proteins from Transformed Cultures of Trichosanthes kirilowii," **Nishant Bhatia**, Karen McDonald, Alan Jackman and Abhaya Dandekar, Annual AIChE Meeting, San Francisco, CA, November 1994.

31. "Thermal and Stress Analyses of Bulk Crystal Growth in a Horizontal Bridgman Furnace," **Greg Young**, Karen McDonald and Ahmet Palazoglu, Poster presented at the Annual AIChE Meeting, San Francisco, CA, November 1994.

32. "Effect of Light on the Production of Sulfolipids Using Cyanobacterial Cultures," **Shivaun Farinha**, Karen McDonald and Alan Jackman, Poster presented at the Annual AIChE Meeting, San Francisco, CA, November 1994.

33. "Effect of Light on the Production of Sulfolipids from Anabaena 7120 in a Fed-Batch Reactor," **Shivaun Archer**, Karen McDonald and Alan Jackman, Fourth Annual Biotechnology Training Grant Retreat, Napa Valley, CA, February 1995.

34. "Anti-HIV Activity of Novel Ribosome Inactivating Proteins," **Karen McDonald**, Alan Jackman, Satya Dandekar, Nishant Bhatia and Carol Oxford, Poster presented at the 12th Annual AIDS Investigator's Meeting, Millbrae, CA, March, 1995.

35. "Effect of Light on the Production of Sulfolipids from Anabaena 7120 in a Fed-Batch Reactor," **Karen McDonald**, Shivaun Archer, and Alan Jackman, Poster presented at the Fifth CIFAR Conference, University of California, Davis, CA, May, 1995

36. "Effect of Growth Morphology on RIP Production from *T. kirilowii* Cultures", Nishant **Bhatia**, Karen McDonald, Dave Reichmuth, Alan Jackman and Abhaya Dandekar, 1995 Annual AIChE Meeting, Miami, FL, November, 1995

37. "Production of Sulfolipids from *Anabaena 7120* in Continuous Culture", **Shivaun Archer**, Karen McDonald and Alan Jackman, 1995 Annual AIChE Meeting, Miami, FL, November, 1995

38. "Characterization of Proteins Secreted by *Trichosanthes kirilowii* Plant Cell Cultures," **N.-J. Remi Shih**, Karen McDonald and Alan Jackman, Poster presented at the Fifth Annual Biotechnology Training Grant Retreat, Napa Valley, CA, April 1996.

39. "On-line Classification of Abnormal/Faulty Process Operations", **Karen McDonald**, James Wong, **A. Palazoglu**, AIGIS System Meeting, Santa Barbara, CA, August, 1996.

40. "Sulfolipid Production from Cyanobacteria in a Continuous, Light-Limited Photobioreactor", **Karen McDonald**, Shivaun Archer and Alan Jackman, Poster presented at the Microbiology Graduate Group Poster Session, October 1996.

41. "Classifications of Process Trends using Hidden Markov Models," **James Wong**, Karen McDonald and Ahmet Palazoglu, Annual AIChE Meeting, Chicago, IL, November 1996.

42. "Production of Plant Defense Proteins from Plant Cell Cultures", **N.-J. Remi Shih**, Karen McDonald and Alan Jackman, Poster presented at the Sixth Annual Biotechnology Training Grant Retreat, Napa Valley, CA, March 1997

43. "*T. kirilowii* Plant Cell Culture in a 5 Liter Bioreactor", **Karen McDonald**, Michael Stoner, N.J. Remi Shih, and Alan Jackman, ACS National Meeting, San Francisco, CA, April 1997.

44. "Production of Plant Defense Proteins from *Trichosanthes kirilowii* Plant Cell Cultures," **Karen McDonald**, N.J. Remi Shih and A. P. Jackman), Poster presented at the Biochemical Engineering X, Kananaskis, Canada, May 1997.

45. "A New Method for Classifying Process Trends Based on Fuzzy Triangular Representation and Hidden Markov Models, **James Wong**, Karen McDonald, Ahmet Palazoglu and Tetsua Wada, IFAC Symposium ADCHEM '97, Banff, Canada, June 1997

46. "Production of Sulfolipids from Cyanobacteria in Photobioreactors", **Seher Dagdiviren**, Karen McDonald, Shivaun Archer, and Alan Jackman), Poster presented at BioHydrogen '97, Kona, Hawaii, June 1997.

47. "Transgenic Tobacco Suspension Cultures for Expression of Ribosome-Inactivating Proteins", **Karen McDonald**, Rajesh Krishnan, Abhaya Dandekar and Alan Jackman, Paper 267e, Annual AIChE Meeting, Miami, FL, November 1998.

48. "Expression of Trichosanthin in Transgenic Tobacco Plants and Suspension Cultures", **Karen McDonald**, Rajesh Krishnan, Abhaya Dandekar and Alan Jackman, ACS National Meeting, Anaheim, CA, March 1999.

49. "Expression of Trichosanthin in Transgenic Tobacco Plants", **Rajesh Krishnan**, Abhaya Dandekar, Alan Jackman and Karen McDonald, Poster presented at the ACS National Meeting, Anaheim, CA, March 1999.

50. "Evaluation of an Optical Technique for Monitoring Biomass in Plant Cell Suspension Cultures", **Shaelyn Hurst**, Karen A. McDonald and Alan P. Jackman, Lasentec FBRM Users Conference, Orlando, FL, Feb 28- March 1, 2000.

51. "'Localization of Recombinant Trichosanthin in Transgenic Tobacco", **Rajesh Krishnan**, Karen McDonald, Abhaya Dandekar, and Alan Jackman, ACS National Meeting, San Francisco, CA, March 2000.

52. "Heterologous Protein Production using Metabolically Regulated Rice", **Melody Trexler**, Karen McDonald, and Alan Jackman, Poster presented at the ACS National Meeting, San Francisco, CA, March 2000.

53. "Studies of Plant Virus Infection in Plant Cell Suspension Cultures", **Masaru Shiratori,** Karen McDonald, Alan Jackman, and Bryce Falk, Poster presented at the ACS National Meeting, San Francisco, CA, March 2000.

54. "Evaluation of an Optical Technique for Monitoring Biomass in Plant Cell Suspension Cultures", **Shaelyn Hurst,** Karen McDonald and Alan Jackman, Poster presented at the ACS National Meeting, San Francisco, CA, March 2000.

55. "Heterologous Protein Production using Metabolically Regulated Rice Cell Suspension Cultures", **Melody Trexler,** Karen McDonald and Alan Jackman, Current Topics in Gene Expression Systems, San Diego, CA, September 2000.

56. "Production of α-1 Antitrypsin from Metabolically Regulated Rice Cell Cultures," **Karen McDonald,** Melody Trexler and Alan Jackman, AIChE Annual Meeting, Los Angeles, CA, November 2000.

57. "A Cyclical, Semi-continuous Process for Heterologous Protein Production Using Metabolically Regulated Plant Cell Suspension Cultures", **Melody M. Trexler,** Karen A. McDonald and Alan P. Jackman, ACS National Meeting, San Diego, CA, April 2001.

Papers presented at Engineering Education Related Meetings:

1. "Building the Confidence of Women Engineering Students: A New Course to Increase Understanding of Physical Devices", D.A. Desrochers, J.M. Henderson, K.A. McDonald, and R. Henes, National WEPAN Conference, Washington, D.C. May 1993.

2. "Exploring the Academic Environment for Women in Engineering", R. Henes, K.A. McDonald, and J. Darby, National WEPAN Conference, Washington, D.C., May 1993.

3. "A Course to Help Build the Confidence of Women Engineering Students", J.M. Henderson, D.A. Desrochers, K.A. McDonald and R. Henes, National Annual ASEE Conference, Urbana, IL, June 1993.

4. "Building the Confidence of Women Engineering Students: A New Course to Increase Understanding of Physical Devices", D.A. Desrochers, J. M. Henderson and K.A. McDonald, Proceedings of GASAT 7 International Conference, Toronto, Canada, July 1993 (presented by V. Cano).

5. "A Multimedia Computer Tutorial Module to Accompany a Bioprocess Engineering Laboratory Experiment", K. A. McDonald, Computer Software Demonstration at NSF Project Showcase, ASEE Annual Conference, Anaheim, CA, June 1995.

Funding:

Extramural

1. American Chemical Society, PRF, Type G "Multivariable Predictive Control of an Ethylene Fractionator," 1987-88, $18,000.

2. University of California, Microelectronics Innovation and Computer Research Opportunities and Harris Microwave Semiconductor, "Elimination of Thermal Stress Induced Dislocations in the Post-Growth Cooling of Crystals," 1987-88, $36,500 (Co-PI Ahmet Palazoglu)

3. National Science Foundation, Research Planning Grant "Production of Alfalfa Somatic Embryos in a Continuous Multiple Stage Bioreactor I. Alfalfa Growth Kinetics," 1988-89, $10,000.

4. University of California, Microelectronics Innovation and Computer Research Opportunities and Harris Microwave Semiconductor, "Elimination of Thermal Stress Induced Dislocations During Growth and Cooling of Single Crystals," 1988-89, $24,000 (Co-PI Ahmet Palazoglu).

5. University of California Microelectronics Innovation and Computer Research Opportunities and Harris Microwave Semiconductor, "Modelling of a Modified Horizontal Bridgman Furnace for Growth of Low Dislocation Single Crystals," 1989-1990, $22,900 (Co-PI Ahmet Palazoglu).

6. National Science Foundation, "Plant Cell Culture Process Development for Production of Trichosanthin," 1990-91, $48,389.

7. University of California Microelectronics Innovation and Computer Research Opportunities and Harris Microwave Semiconductor, "A Modified Horizontal Bridgman Furnace for Growth of Low Dislocation Single Crystals-Growth, Analysis and Modeling," 1990-91, $26,265 (Co-PI Ahmet Palazoglu).

8. National Science Foundation, Research Experiences for Undergraduates, Supplement to "Plant Cell Culture Process Development for Production of Trichosanthin," 1990-91, $10,500.

9. National Science Foundation, "A Model Undergraduate Project for Women in Engineering," Grant # HRD 9053903, 6/15/91-11/30/94, $69,997, PI: K. McDonald.

10. University of California Systemwide Biotechnology Research and Education Program, Planning Grant, "Production of Sulfolipids Using Cyanobacterial Cultures," 7/1/93-6/30/94, $15,000.

11. USDA National Needs Graduate Fellowships Grants Program, "Bioprocess Engineering of Agricultural Resources for High Value Products," Grant # 93-38420-8805, 7/27/93-6/30/97, $108,000 (PI: D. Ryu, co-PI's: K. McDonald, A. Dandekar, D. Durzan, R. Garrett, A. Jackman, B.J. McCoy, R. Merson, D. Ogrydziak, R. Rodriguez).

12. Ensuiko Sugar Refining Company, Gift, $5,407, 4/94.

13. Unocal Foundation, "Interactive Computer Simulations of Petrochemical Processes: A Teaching Tool for Engineers and Nonscientists," 4/94-12/95, $30,000, Co-PI's: K. McDonald, A. Palazoglu.

14. National Science Foundation, "A Collaborative Laboratory Course on *How Things Work* Designed to Improve Retention of Women in Engineering," Grant # DUE-9354539, 7/1/94-6/30/95, $116,456, PI: J. Henderson, Co-PI's: K. McDonald, J. Darby.

15. University of California Systemwide Biotechnology Research and Education Program, "Production of Sulfolipids Using Cyanobacterial Cultures," 7/1/94-6/30/97, $120,000, Co-PI's: K. McDonald, A. Jackman, J. Meeks, B. German.

16. University of California Universitywide AIDS Research Program, "Anti-HIV Activity of Novel Ribosome Inactivating Proteins," Grant # R94-D-079, 7/1/94-6/30/96, $50,200, PI: K. McDonald, Co-PI's: A. Jackman, S. Dandekar, A. Dandekar, S. Cohen.

17. National Science Foundation, "Leadership Projects in Laboratory Development: A Bioprocessing Laboratory," Grant # DUE-9451339, 9/1/94-8/31/97, $100,000 (with $100,00 non-state matching funds from the University), PI: K. McDonald, Co-PI's: N. Abbott, L. Bisson.

18. Chevron Research and Technology Company, "Intelligent Process Monitoring & Diagnosis," 9/1/94-3/31/98, $21,000, PI: A. Palazoglu, Co-PI: K. McDonald.

19. National Science Foundation, "A Novel Cell Line and Improved Plant Cell Culture Strategies for the Production of Ribosome Inactivating Proteins," Grant # BCS-9407177, 9/15/94-11/31/97, $234,010 (with $27,700 equipment matching funds from the University), Co-PI's: K. McDonald, A. Jackman and A. Dandekar.

20. National Science Foundation, Research Experiences for Undergraduates Supplement to "A Novel Cell Line and Improved Plant Cell Culture Strategies for the Production of Ribosome Inactivating Proteins," BCS-9407177, 9/15/94-8/31/96, $10,000, Co-PI's: K. McDonald, A. Jackman and A. Dandekar

21. Aigis Systems Inc., 1/21/97, Gift, $30,000

22. National Science Foundation, Research Experiences for Undergraduates Supplement to "A Novel Cell Line and Improved Plant Cell Culture Strategies for the Production of Ribosome Inactivating Proteins, " BES 9407177, 6/15/97-11/31/97, $10,000, Co-PI's: K. McDonald, A. Jackman and A. Dandekar.

23. National Science Foundation, "POWRE: A Plant Cell Suspension/Plant Viral System for Expression of Heterologous Proteins," BES 9870453, 8/15/98-1/31/00, $74,971, PI: K. McDonald

24. University of California BioSTAR and Applied Phytologics, Inc, "Novel Operating Strategies for Continuous Production of Recombinant Molecules Using Regulated Rice Cell Cultures", S98-04, 6/1/99-3/31/02, $132,979, PI: K. McDonald, CoPI: A. Jackman

25. National Science Foundation, "PGE/PG: Planning the Central Vallley Community Science Project for Gender Equity in Culture, Climate and Curriculum", HRD 9976255, 7/1/99-6/30/00, $30,000, PI: B. Holmen, CoPI's: K. Longworth, K. McDonald and J. Utts.

26. USDA NRICGP, "Extracellular Targeting and Improved Recovery of Proteins from Transgenic Tobacco", #99355047798, 9/1/99-8/30/02, $160,000, Co-PI's: K. McDonald, A. Jackman and A. Dandekar

27. University of California BioSTAR Executive Committee Initiative, "Meetings to Increase Communication and Research Partnerships Between Campus Researchers and California Companies, S98-302, "10,000, 9/15/99-5/30/01, PI: K. McDonald

28. University of California BioSTAR Executive Committee Initiative, "Meetings to Increase Communication and Research Partnerships Between Campus Researchers and California Companies, $15,000, 6/30/01-6/30/02, PI: K. McDonald

29. University of California BioSTAR, California Dairy Research Foundation and RZ Syntopical Technologies, "Engineering Probiotic Microorganisms", Grant# 01-10204, $1,137,874, 8/13/02 - 8/12/06, PI: D. Mills, Co-PIs: D. Block, K. McDonald, B. Lonnerdal, L. Gershwin

30. National Science Foundation, "A Regulated Plant Virus Expression System for Efficient Production of Human Therapeutics", BES 0214527, $755,013, 9/1/02-8/31/05, PI: K. McDonald, Co-PI's: A. Jackman, A. Dandekar and B. Falk

31. California Department of Food and Agriculture, "Design of Chimeric Anti-microbial Proteins for Rapid Clearance of Xylella", $198,000, 7/1/03-6/30/04, PI: A. Dandekar, Co:PI's: G. Gupta, K. McDonald, E. Hong-Geller, P. Unkefer, C. Unkefer

32. University of California Discovery Grants, Opportunity Award, "Engineering Challenges in the Post-Genomic Era", Grant #02-212, $7,700, PI: K. McDonald, Co-PI's: R. Powell, D. Block, B. Higgins

Intramural

1. Faculty Research Grant, "Application of Dynamic Matrix Control to Nonlinear Processes," 1986-87, $3,000.

2. Faculty Research Grant, "Dynamic Modelling of Fermentation Processes," 1987-88, $3,000.

3. Undergraduate Instructional Improvement Grant, "Modification of the Distillation Experiment in the Unit Operations Laboratory," with A. Palazoglu, 1987.

4. Undergraduate Instructional Improvement Grant, "Automation of the Evaporation Experiment in the Chemical Engineering Unit Operations Laboratory," with A. Palazoglu and B.W. Bequette, 1988, $3,700.

5. Faculty Development Award, 1988-89, $1,000.

6. Faculty Research Grant, "Modelling of Oscillations in Continuous Cultures of *Saccharomyces cerevisiae*," 1988-89, $1,500.

7. Faculty Research Grant, "Modelling of Oscillatory Behavior in Continuous Cultures of *Saccharomyces cerevisiae*," 1989-90, $1,800.

8. Junior Faculty Research Grant, "Development of Analytical Techniques for Determination of Trichosanthin in *T. kirilowii* Callus and Plant Tissues," 1990-91, $2,000.

9. Faculty Research Grant, "Dynamic Modelling of Continuous Fermentation Systems," 1990-91, $2,000.

10. Faculty Research Grant, "Analysis of Phospholipids and Sulfolipids in Algal Cultures," 1991-92, $1,950.

11. Faculty Research Grant, "Modelling of Thermal Stress During Bulk Single Crystal Growth," 1992-93, $2,300.

12. Faculty Research Grant, "Dynamics of Growth and Protein Production in Plant Cell Cultures", $2,000, 7/1/93-6/30/94.

13. Faculty Research Grant, "Ribosome Inactivating Protein Activity from Plant Cell Suspension Cultures," $1,500, 7/1/94-6/30/95

14. Undergraduate Instructional Improvement Fund, "Interactive Computer Simulations/Games for Integrated Learning, Grant # U93-109, 1/6/94-6/30/94, $5,000, Co-PI's: K. McDonald, A. Palazoglu.

15. Faculty Research Grant, "Initiation of Plant Cell Cultures of *Marah Oreganus* for Production of Ribosome Inactivating Proteins", $1,500, 7/1/97-6/30/98.

16. Teaching Resources Center, Minigrant, "Biotechnology Industry Representative's Visit", $157.93, 7/30/97-6/30/98

17. Office of the Vice Chancellor for Research, Bridge Grant "Plant and Cyanobacterial Cultures for Production of High Value Compounds", $20,000, 3/98-2/99.

18. College of Engineering, Sloan Grant Award for new, introductory laboratory course entitled "Exploring the Interface Between Chemical Engineering and Materials Science", taught as ECH98 in Spring 98, $5,000, 9/97-8/98.

19. Faculty Research Grant, "Role Of Plant Viral Coat Protein On Elicitation Of Ribosome Inactivating Proteins", $1,500, 7/1/99-6/30/00

Patent Disclosures:

"Plant Cultures Producing Ribosome Inactivating Proteins," (with J. Thorup, A.P. Jackman and A. Dandekar), U.C. Case #92-303, November 1992.

"Process for Scaled-up Production of Recombinant Proteins Using Transgenic Plant Suspension Cultures" (with M. Trexler and A. Jackman), UC Case # 2001-083, November 2000. Patent Application filed Nov 14, 2001.

TEACHING

Courses Taught:

Process Dynamics and Control, ECH 157 (Fall-1985-87, 1990, 1995, Spring-91)
Process Control Laboratory, ECH 157L (Winter 1992, 1993, Spring 1994,Winter 1995, Spring 1996, Winter 97, Winter 2003)
Chemical Engineering Laboratory, ECH 155A/B (Winter-1986-88, 91, Fall-1989, 1991, 1994,Winter 1994)
Biochemical Engineering Fundamentals, ECH 161 (Spring-1986-93)
Biochemical Engineering Fundamentals, ECH161A (Winter 1994, 1996, 2000)
Bioprocess Engineering Laboratory, ECH161L (Spring 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003)
Chemical Engineering Kinetics, ECH 156B (Spring 1992, 1993)
Economics and Optimization of Chemical Processes, ECH 158A (Fall 1992, 1997, 1999, 2000, 2001, 2 lectures in Fall 2003)
Plant Design, ECH158C (Spring 2000)
Principles of Biomanufacturing, ECH198 (Winter 2004)
Biochemical Engineering, ECH206 (Winter 1996, 1998, 2000)
Advanced Process Control, ECH 267 (Spring-1987-88)
Advanced Heat Transfer, ECH 253B (Winter-1989, 1990)
Biotechnology Fundamentals and Application, MCB263 (Winter 2001, co-taught with Rosemary Smith and Marty Privalsky, Winter 2003, co-taught with Ray Rodriguez)
Introduction to Physical Devices and Systems, ENG25 (Winter 1995)
Exploring the Interface Between Chemical Engineering and Materials Science, ECH98 (Spring 1998)
Fermentation Science, VEN 186 (Spring 1994, 1996)
2-3 Lectures in Fermentation Science, VEN 186 (Spring 1989, 1991)
Seminars in Process Dynamics and Control, ECH 292 (Winter-1987-90)
Coordinator for Chemical Engineering Seminar, ECH 290 (Winter 1993, Spring 1994, Spring 1997, Spring 2001)
Coordinator for Chemical Engineering Graduate Student Seminar, ECH 193 (Fall 1997)
Coordinator for Introduction to Chemical Engineering, ECH1 (Winter 1995, 1996, 1997)
1 Lecture in Research Opportunities in Engineering, IST9 (Spring 1993, 1994, 1995, 1996, 1997,1998, Fall 2002)
1 Lecture in Principles and Concepts of Plant Biotechnology, PLS 160 (Winter 1994-2003)
1 Lecture in Recombinant DNA and Genetic Engineering, MCB 262 (Winter, 1999)
3 Microbiology Lab Rotation Students, MIC 201L (Fall 2000 - Jeff Reidmiller, Fall 2003 - Paula Mara)

Teaching Evaluations:

In response to "overall evaluation of instructor" my yearly averages on a scale from 1 (low) to 7 (high) over the past 5 years are:

1997-98	6.38
1998-99	6.37
1999-00	6.59
2000-01	6.32
2001-02	6.56
2002-03	5.81

Course/Curriculum Development Improvements:

1. Received two campus Undergraduate Improvement Grants for improving the undergraduate Chemical Engineering Laboratories:

   i) "Modification of the Distillation Experiment in the Unit Operations Laboratory," with A. Palazoglu, 1987.

   ii) "Automation of the Evaporation Experiment in the Chemical Engineering Unit Operations Laboratory," with A. Palazoglu and B.W. Bequette, 1988.

2. Revised Process Control course structure and laboratory experiments.

3. Introduced a pilot scale fermentation, laboratory scale fermentation, oxygen transfer experiment and computer modelling project in the Biochemical Engineering course, ECH 161.

4. Introduced the oxygen mass transfer experiment in ECH 155B, Winter 1994.

5. Designed curriculum for new major in Chemical/Biochemical Engineering. Wrote proposal for new major and presented the proposal to faculty at College faculty meeting. New major was approved in May, 1994.

6. Prepared and submitted a proposal to NSF, Course and Curriculum Development on "A Collaborative Laboratory Course on *How Things Work* Designed to Improve Retention of Women in Engineering" (with J. Henderson and J. Darby), June 1993

7. Wrote and submitted a proposal to the Unocal Foundation on "Interactive Computer Simulations of Petrochemical Processes: A Teaching Tool for Engineers and Nonscientists," (with A. Palazoglu), September 1993

8. Prepared and submitted a proposal to NSF, Leadership in Laboratory Development program, "Development of a Multidisciplinary Bioprocessing Laboratory that uses Multimedia", (with Nick Abbott and Linda Bisson), November, 1993

9. Prepared and submitted a proposal to NSF, Course and Curriculum Development program (with A. Palazoglu) "A Novel Course on Issues and Options In Manufacturing: Bridging the Gap Between Science and Nontechnical Students", June 1994

10. Participant in UC Davis Summer Institute on Technology in Teaching, July 1995.

11. Attended the "Educational Environment for Women in Engineering, Mathematics and Science" Fall Faculty Retreat, October 4-5, 1997.

12. Introduced a new, hands-on inquiry-based introductory laboratory course (with M. Meier) entitled "Living in a Material World: Exploring the Interface Between Chemical Engineering and Materials Science", Spring 98, Funded through the College of Engineering Sloan Grant

Undergraduate Research:

I have supervised the following students in undergraduate research projects (ECH199, PUFF program, NSF REU, MORE program, McNair Scholars program, NSF LURE, etc, an asterisk indicates that the student went on to graduate school):
Mark Johnson*, Darrell Lewis-Sandy, Sara Stokes, Monica Gomes, Richard Cheung, Caroline Crispino, Tuyen Do, Yvette Mangaladin, Anh Nguyen, Mondana Panymanian, Eunice Tan, Ruth Chan (MORE), Arlene Adela, James Fred Johnson*, Michelle Bishop*, Rachael Balog, Dave Reichmuth*, Casey Bunker*, Cynthia Buenviaje*, Michael Costello, Nicole Dennis, Richard St. John*, Tracy Horsfield, Yea-Lian Huang, Linda Leong, Khan Nguyen, Katrina Meehan, David Mills*, Phuong Anh Nguyen, Bill Ristenpart* (PUFF), James Corpuz, Chris Humphrey, Michael Stoner*, Devin Eselius, Eunice Tan, Danielle Coutts, Shaelyn Hurst (McNair Scholar), Ruth Chan, Christine Owlett*, Shane Hall, Annie Chou (MORE), Megumi Noguchi, Tammy Adamson (LURE), James Corpuz, Phillip Tuet, Jolie Wu, Mark Sanford, Andrew Tung, Diemchi Nguyen, Jennifer Mikovich, Jeff Luk*, Ming-Wai Chen, Debby Kolbeck, Sandra VanDusen, Kali Campbell, Jennifer Chen, Alison Hong, David Trombly, Javier Lacayo, Nathan Parker, Drew Erlich.

Several of my undergraduate students have presented their work at the UC Davis Annual Undergraduate Research Conference:

William Ristenpart, "A Study of the Effects of Plant Virus and Plant Virus Coat Protein on Ribosome Inactivating Protein Production in Plant Cell Suspension Culture", April 1999

Christine Owlett, "Sedimentation of Plant Cell Aggregates", April 1999

Ruth Chan, "The Study of Fatty Acid Composition in Sulfolipids", April 1999.

Jeff Luk, "Kinetics of Trichosanthin Production from Transgenic Tobacco", April 2001

Mark Sanford, "Trichosanthin Protein Recovery from Transgenic Tobacco Plant Cell Culture using Column Chromatography", April 2002

Sandra Van Dusen, "Comparison of Recombinant α-1-antitrypsin Production from Rice Cell Cultures in Shake Flasks and Membrane Bioreactors", April 2002

Lo Ming Hong and David Trombly, "Transgenic Rice Cell Production of Alpha-1-Antitrypsin in Shake Flask and CELLine Bioreactor, April 2003

Lo-Ming Hong and David Trombly, poster presentation "Use of a Membrane Bioreactor for Production of Alpha-1-Antitrypsin in Transgenic Rice", National Undergraduate Research Conference, April 2004.

Several of my undergraduates won First Place in the AIChE Western Regional Student Paper Competition based on their undergraduate research projects (Mark Johnson, Chris Humphrey, Michael Stoner and Danielle Coutts)

I was also nominated for the Chancellor's Award for the Mentoring of Undergraduate Research, June 1999

Research Advisor for:

Student	Co-Advisor	Degree Awarded/Expected (Thesis/Dissertation Title)	Current Position
Ching I. Chen		Ph.D., Microbiology Graduate Group, September, 1991 *Analysis and Modelling for the Oscillatory Behavior of Saccharomyces Cerevisia in a Continuous Bioreactor System*	AlleCure, Inc. Senior Scientist Biologic Therapeutics Valencia, CA
Michael Cooney		Ph.D., Chemical Engineering, June 1992 *Multivariable, Dynamic Model Development for Pure Continuous Cultures of E. Coli and C. Utilis*	Associate Professor Dept. of Ocean and Resources Engineering Marine Bioproducts Engineering Center University of Hawaii at Manoa Honolulu, Hawaii
Michael Lucas	A. Palazoglu	M.S., Chemical Engineering, December 1988	Dow Chemical Company Pittsburg, California
John Thorup	A. Jackman	M.S., Chemical Engineering, December 1992 *Purification and Identification of Ribosome Inactivating Proteins from Plant Cell Cultures of Trichosanthes Kirilowii*	Dow Chemical Company Contract Manufacturing Services - R & D Midland, Michigan
Greg Young	A. Palazoglu	Ph.D., Chemical Engineering, 1993 *Thermal and Stress Analysis of Single Crystal Growth in a Horizontal Bridgman Furnace*	Assistant Professor Chem. Engr. Dept San Jose State University San Jose, CA
Annette Hagewiesche	A. Jackman	M.S., Chemical Engineering, 1991 Plan II	Genentech, Inc. Recovery Sciences (Development/Process Sciences) 1 DNA Way South San Francisco, CA 94080-4990
Yen Ko	A. Jackman B. McCoy	M.S., Chemical Engineering, 1993 *Cyanobacteria Photoproduction in a Stirred Reactor*	
Kirti Patel	A. Palazoglu	M.S., Chemical Engineering, 1993 Plan II	Intel Corporation
Iftikar Gohar	A. Palazoglu	M.S., Chemical Engineering, 1993 *Numerical Simulation of Natural Convection in Liquid Semiconductor Metals in Modified Bridgman Furnace*	Van den Bergh Foods, Columbia, Maryland

Shivaun Archer	A. Jackman	Ph.D., Chemical Engineering, 1996 *Effect of Light on the Production of Sulfolipids from Cyanobacteria*	Research Associate Department of Chemical Engineering Cornell University Ithaca, NY
Nishant Bhatia	A. Jackman	PhD, Chemical Engineering, 1997 *Ribosome Inactivating Proteins from Trichosanthes kirilowii Cultures*	Diosynth, RTP Associate Director, Validation Cary, NC
Seher Dagdevirin	A. Jackman	M.S., Chemical Engineering,1999 *Sulfolipid Production from the Cyanobacterium Synechocystis sp. PCC 6803 in Fed-Batch Photobioreactors*	Bayer Corporation Pharmaceutical Division Quality Assurance Associate Scientist Berkeley, CA
James Wong	A. Palazoglu	PhD, Chemical Engineering, 1998 *Classification of Process Trends Based on Fuzzified Symbolic Representation and Hidden Markov Models*	OSI Software Software Developer San Leandro, CA
Rajesh Krishnan	A. Jackman	PhD, Chemical Engineering, 2000 *The Expression, Localization and Characterization of Recombinant Trichosanthin, a Type I Ribosome-Inactivating Protein, in Transgenic Tobacco*	Pfizer, Inc. Biologics Development Group Research Scientist Groton, CT
Masaru Shiratori		PhD, Chemical Engineering Expected 2003	
Melody Trexler	A. Jackman	PhD, Chemical Engineering January, 2003 *A Cyclical Semi-Continuous Process for Production of Heterologous Proteins in Metabolically Regulated Plant Cell Suspension Cultures*	Associate Scientist Genentech, Inc. Recovery Sciences (Development/Process Sciences) 1 DNA Way South San Francisco, CA 94080-4990
Christine Owlett	A. Jackman	MS, Chemical Engineering December 2002 *Therapeutics from Plant Cell Suspension Cultures*	
Sinyoung Park		PhD, Chemical Engineering September, 2003 *Mammalian Cell Respiration and O_2 /CO_2 Transfer in High-density Perfusion Culture with Microsparge Oxygenation and On-line Mass Spectrometry*	Bayer Corporation **Title?**
Liz Zapalac		MS, Chemical Engineering March 2003 *Purification of Alpha-1-Antitrypsin using a Three-Step Chromatography Process - Factors Effecting Dynamic Binding Capacity, Production Rate and Costs*	Bayer Corporation Requal Engineer Berkeley, CA

MS/PhD Dissertation Committees:

Sun Ho Park, PhD ChE, "Optimization of Monoclonal Antibody Production in Hybridoma Cell Culture: Biomolecular and Bioprocess Engineering Approaches", August 1993
Keith Fruzzeti, PhD ChE, "Studies on Linear and Nonlinear Model Predictive Control of Chemical Processes", 1994
Rabia Ballica, PhD ChE,
Eric Hanczyc, PhD ChE, "Modeling and Control of Chemical Process Systems Described by Partial Differential Equations", Jan. 1994
Ming Wang, PhD ChE, "Chemical Kinetics of Complex Systems: Thermal Degradation of Coal & Polymers", August 1994
Mary Quasney, MS Nutrition, "In-Vitro Effects of Sulfoquinovosyldiacyl-Glycerol (SQDG) on Gastric and Colonic Cancer Cells", 1999
Rajesh Krishnan, MS ChE, "Performance of an Ultrafiltration Hollow Fiber Reactor for Enzymatic Synthesis of Phospholipids Coupled with Cofactor Regeneration", 1999.
Kurt Ohlinger, PhD CEE, "Struvite Controls in Anaerobic Digestion and Post-digestion Wastewater Treatment Processes", 1999
Brian Argo, MS ChE, "Adsorption Kinetics of Soluble Surfactants on Model Thiol Surface", 2000
David Cipolla, MS ChE, "An Analysis of the Kinetics and Mechanism of the Cephalexin synthesizing System Utilizing Purified Acetobacter turbidans Enzyme", Sept. 2000
Ayse Batigun, PhD ChE, "Analysis of Nonlinear Systems Using Functional Expansion", January 2001.
Liqin Zhang, PhD ChE, "Mobile Phospholipid Bilayers on a Polyion/Alkylthiol Layer Pair as a Biosensor Model for Selective Peptide Detection", May 2001
Matthew Olsen, MS ChE, "Development of a Novel Process Optimization Technique Utilizing a Hybrid Statistical-Artificial Intelligence Approach", July, 2001
Wei Sun, PhD ChE, "Process Trend Analysis via Wavelet Domain Hidden Markov Models," November, 2001
Fuat Doymaz, PhD ChE, "Statistical Monitoring and Modeling of Multivariable Systems," January, 2002
Chen Ma, MS ChE, "Lifting-up of Supported Lipid Bilayers on Layer-by-Layer Polyion/Alkylthiol Cushions", March 2002.
Satyam Godasi, PhD ChE, "Identification and Control of Non Linear Distributed Parameter Systems" May, 2002.
Melinda Jones, MS Biological Systems Engineering, "An Economic Analysis of Alpha-1-Antitrypsin Production in Rice Cells and Rice Seed", October 2002

PhD Oral Qualifying Exam Committees last five years:

Shuman Mitra, April 1995
Raj Krishnan, June 1996
Greg Mayeur, Sept. 1996
Ken Harris, May 1996 (chair)
Hanshu Ding (Food Science), 1996
Ling Wang, March 1997
Christine Smith, May 1997
Kurt Ohlinger (Civil and Environmental Engineering), Nov, 1997
Fuat Doymaz, Dec, 1997 (chair)
Wei Sun, May 1999
Satyam Godasi, May 1999 (chair)
Young-Son Sohn (Microbiology Graduate Group), March 1999
Hung Ly, June 2000
Jae-Han Kim (Food Science), December 2001
Matthew Coleman, June 2002
Tian Bao, February 2003
Larry Joh (Bilogical Systems Engineering), July 2003

SERVICE

Administrative Service as Associate Dean

Served as College liaison to University Outreach and International Programs including serving on the International Programs Advisory Council (2001,2002), Reviewer for Seed Grants (2001,2002), facilitating General Agreements of Cooperation and International Working Agreements with other Universities and Research organizations.

Provide input to Dean on Graduate related issues such as proposals for new graduate programs (PhD program in Linguistics in 2002), nominations for faculty to serve on internal fellowship review

Professional:

- Finance Chair for 1992 American Control Conference.
- Chaired sessions at 1986 (2), and 1988, 1990, 1991 AIChE Meetings, 1991 ACC Meeting, 1992, 1993 ACS Meeting, 1997 Biochemical Engineering X poster session, 1997 AIChE National Meeting, 2000 ACS National Meeting, 2001 ACS National Meeting.
- Reviewer for AIChE Journal (11), I&EC Research (6), IFAC (2) Chemical Process Control III (1), American Journal of Enology & Viticulture (4), Chemical Engineering Communications (2), International Journal of Control (3), Numerical Heat Transfer (1) American Control Conference (5), Biotechnology Progress (6), Biotechnology and Bioengineering (8), Protein and Peptide Research (1), Canadian Journal of Chemical Engineering (2), IFAC (1), ADCHEM 97 (1) Journal of Biotechnology (1), Applied Biochemistry and Biotechnology (2), External Examiner for PhD Dissertation, University of New South Wales, FEMS Microbiology Letters (1)
- Reviewer for NSF (13 proposals and 6 panel reviews: 1995 Combined Research/Curriculum Development Concept Paper, 1996 CAREER panel, 1997 CCD panel, 1999 SBIR/STTIR panel, 2001 BES Large Proposals Panel), UC Energy Research (2), American Vineyard Foundation (1), USDA (2), San Diego Super Computer (1), Frontiers in Bioprocessing III, (2), State of Louisiana Board of Regents (1), UC Biotechnology Program(3), Ted Peterson Student Paper Award (1996), UC Biotechnology STAR Proposal Review Panel (1996-2002), Evaluation letters for tenure candidates (2), Proposal reviewer for Research Grants Council, Hong Kong (2001,2002, Outside evaluation letter for promotion (1).
- Consultant for OEA Aerospace, Fairfield, CA 1998

Presentations/University and/or Community Service:

1) Presentations at local elementary schools on engineering as part of "A Day with Women Who Love Science."

2) Presentation at Emerson Jr. High Career Day, Davis, CA, April, 1986.

3) Panelist for Expanding Your Horizons Conference, Solano Community College, Suisun, CA, March 1986.

4) Organizer and Panel Discussion leader for a Workshop on Graduate Studies in Engineering, SWE Western Regional Conference, April, 1987.

5) "Out in the World: The Engineering Option," Athena Meets Prometheus: Gender, Science and Technology, University of California, Davis, CA, April 1988.

6) "Graduate Study in Engineering," Sacramento Valley Section of Society of Women Engineers, Sacramento, CA, Nov. 1988.

7) Career and Family Panelist, Society of Women Engineers National Conference, Oakland, CA, June, 1989.

8) Should You Consider a Career in Academia Panelist, "Technical Opportunities in the 90's," CSUS Society of Women Engineers and CSUS Women's Programs, California State University, Sacramento, CA, February 1990.

9) "Combining an Academic Career with Child Raising: Is There Ever a Good Time?" Panelist, UCD Network for Graduate and Faculty Women, University of California, Davis, CA, March 1990.

10) Presenter and Planning Committee Member for Faculty Workshop on "Exploring the Academic Environment for Women in Engineering," 1991-92

11) Judge, 39th Annual California Central Valley Science & Engineering Fair, April, 1992.

12) Co-organizer for Workshop on "Exploring the Academic Environment for Women in Engineering," UC Davis, May 1992.

13) Steering Committee Member for Workshop on "Exploring the Academic Environment for Women in Engineering, UC Davis, 1992-1993

14) Speaker at the Women in Engineering Conference at Berkeley on "The Undergraduate Experience: Surviving and Thriving", UC Berkeley, October, 1993

15) Presentation at Colloquium for High School Students sponsored by Clorox/Dept. of Chemical Engineering, April 1994, 1995 (Lab project)

16) SWE Little Sisters Day Faculty Panel, UC Davis, June 1994

17) Women's Engineering Link Faculty Panel, UC Davis, April 1995.

18) Laboratory Tour, SWE Little Sister's Day, UC Davis, April 1995, 1996.

19) Laboratory Tour, Overview of Biochemical Engineering and SimRefinery Demonstration, Colloquium for High School Students sponsored by Clorox/Dept. of Chemical Engineering, April 1995.

20) "Plant Cell Culture Production of Antiviral Proteins: Engineering and Medicine Working Together," Sacramento Valley Section of Society of Women Engineers, Davis, CA, May 1995.

21) "Plant Cell Culture Production of Antiviral Proteins: Engineering and Medicine Working Together," AIChE Student Chapter, University of California, Davis, CA May 1995.

22) "Graduate School," Panelist, Society of Women Engineers, Davis, CA, Jan 1996.

23) Bioprocess Engineering Laboratory Tour, Picnic Day,UC Davis, April 1996

24) Influence of Fluid Shear on Insect Cells Laboratory for Clorox Company sponsored High School Colloquium, UC Davis, April, 1996.

25) Balancing Family and Career Panel, Women's Engineering Link, UC Davis, May, 1996.

26) Panel presentation on "Higher Education in the United States", New International Students Orientation, UC Davis, Sept. 1997.

27) Workshop Presenter, "Making the Bounciest Ball", Expanding Your Horizons Conference, UC Davis, March 2000.

28) Workshop Presenter, "Grant Writing in the Sciences", Dissertation-Year and Presidential Postdoctoral Fellowship Program, Fall Conference, Oakland, CA, September 2000.

29) Moderator, UC Davis Undergraduate Research Conference, April 2001, 2002.

30) FIRST (For Inspiration and Recognition of Science and Technology) Robotics Team Mentor and Advisor, Vanden High School Robotics Team 701, 2002

31) Panel Presentation, "Graduate Recruitment Strategies", Chancellor's Fall Conference, September 2003.

University:

Member of the Microbiology Graduate Group
Member of the Fermentation Science Undergraduate Group
Co-Director of the NIH Graduate Training Program in Biomolecular Technology, Fall 2003-
Faculty Trainer, NIH Graduate Training Program in Biomolecular Technology, 2000-
Status of Women at Davis Administrative Advisory Committee, 1986-89, 1994-95
  Academic Subcommittee Member
  Co-chair of Childcare Subcommittee (Co-author of SWADAAC Report
  "Childcare Concerns, Problems and Needs of UCD Students")
Educational Opportunity Program/Student Affirmative Action, 1988-89
Chancellor's Student Affirmative Action Achievement Award Committee, 1990
Presentation for Legislative Analyst Visit, 1990
Committee on Preparatory Education, 1991
Ad-hoc Committee on Graduate Student Environment, 1993
Outstanding Graduate Student Teaching Award Committee, 1993
Faculty Search Committee, Biochemical Engineer Position in Viticulture and Enology, 1993,1994
Faculty Search Committee, Biological Systems Engineer Position in Biological and Agricultural Engineering, 1995
Selection Committee, Director of Biotechnology Program at UC Davis, 1994
Ad-Hoc Committee (7), 1993,1995, 1997, 2002
Committee on Undergraduate Scholarships, Honors and Prizes, 1994-1995
University Medalist Interview Committee, 1995
Representative for the Academic Senate 1995 (perfect attendance record!), 1996, 1997

Advisory Committee for the Campus Biotechnology Program 1996
University of California Biotechnology STAR Program, Executive Committee Member 1996-01
University of California Forest Products Laboratory Technical Advisory Committee Jan 1997- Dec 1999
Committee on Educational Policy 1997
Student-Faculty Relationships Committee, 1999, 2000, 2001
Recruitment Committee, Director of Technology Transfer, 1998
Designated Emphasis in Biotechnology Faculty Member and Executive Committee Member 2001-03
Faculty Mentor for Orit Kalman, President's Postdoctoral Fellow, 2000
Recruitment Advisory Committee for Dean, College of Engineering 2000, 2001
Advisory Board Member, Consortium for Women and Research 2001-03, Reviewer for Graduate Travel Awards, Faculty Mentor Awards, Graduate Research Awards
University Outreach and International Programs Advisory Board 2002-
Dean's Council of Graduate Affairs, 2000-
Publication and Security Task Force, 2002
Internal Program Task Force, 2002

College:

Graduate Study Committee, 1993, 1994 (Chair), 1995-97, Ex-officio member 2000-
Co-Advisor for Society of Women Engineers Student Chapter, 1987-93
Dean's Biotechnology Advisory Committee, 1986-88
Ad Hoc Committee on Women in Engineering, 1989-90
Student Affirmative Action, 1990-1992
Engineering Summer Residency Program Participant, 1990,1991
Summer Advisor, 1990, 1991
Industry Advisory Board for the Center for Women in Engineering, 1992-97
Tau Beta Pi Advisory Board Member, 1992-95
Judge, SWE/Varian Technical Paper Competition, 1993
Staff Incentive Awards/Staff Recognition and Achievement Awards Committee 94-96
Subcommittee on Proposed Integrated BS/MS Plan, 1996
College Student Diversity Committee 1996, 1997
Search Committee, Anheuser Busch Chair in Brewing Sciences, 1997
Distinguished Engineering Alumni Award Selection Committee, 1997, 1998
Committee on Student Development, 1998, 1999 (Chair)
MORE Advisory Board 2002
Kemper Hall Naming Committee, 2002
Dean's Advisory Committee, 2000-

Departmental:

Advisor for AIChE Student Chapter, 1985-87
PhD Preliminary Exam Committee, 1987-89, 1993
Undergraduate Scholarship Committee, 1986-89, 90, 91, 92-93(chair)
Faculty Search Committee, 1990-91, 95
Advisor for ~ 40 undergraduates, 1985-1994
Ad-hoc Committee on the Future, 1992
Graduate Advisor, Winter, Spring 1993, 1994-95, Winter-Spring 96, 97
Graduate Admissions Committee, 1994-95
Graduate Awards Committee, 1994-95
Undergraduate Curriculum Committee (2000 (Chair), 2001, 2002)